

# ŒNOIVAS 2019

# BOOK OF ABSTRACTS

**Oeno 2019**  
*11<sup>th</sup> International Symposium  
of Enology of Bordeaux*

11<sup>th</sup> edition

**IVAS 2019**

In Vino  
Analytica  
Scientia

Analytical Chemistry for wine,  
Brandy and Spirits

From the 25th to the 28th of June

**25-28**  
June 2019

**ŒNOIVAS 2019**  
INP/ENSEIRB Matmeca,  
avenue des Facultés,  
33405 Talence



# ENOIVAS 2019



<https://isvv-events.com/oen2019-ivas2019>



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## Wine should only be wine

People trust their wine to be exactly as they expect. Unadulterated, safe, consistent. And not coincidentally, each of the world's top ten beverage companies trust us to help keep it that way. They know how long it takes to build a brand's reputation and how quickly one can disappear. So they come to us and our widest instrumentation portfolio on the planet — ion, liquid and gas chromatography, metal analysis, mass spectrometry, discrete analyzers to data management — for accurate, reliable answers no matter where they are in the beverage analysis process. They trust us to help keep their product their product. And equally important, their brand their brand.

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# Local Organizing Committee

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## Welcome to the participants of the joint congress **EnoIVAS 2019** **11th International Symposium of Oenology of Bordeaux** **and 11th edition of the symposium In Vino Analytica Scientia**

On behalf of the Oenology Research Unit at ISVV, we are pleased to welcome you in Bordeaux for 4 days of scientific work presentation and exchanges as well as for pleasant moments in the reception hall of Bordeaux INP and on Garonne docks, in the Cité du Vin and then nearby in Faïencerie, lobby for the Gala dinner.

You are nearly 350 delegates, mainly scientists, from 24 countries to discuss as well as to exchange different aspects of wine and spirits research. We also hope that you will visit our beautiful Bordeaux city, whose docks have been listed as a World Heritage Site.

This congress would not have been possible without the support of the Conseil regional de la Nouvelle Aquitaine (New Aquitaine council), IDEX of Bordeaux University, Bordeaux INP, Institute of wine and vine science and Department of Environmental Sciences from Bordeaux University, as companies sponsoring us : Thermo Fisher, Gerstel, BioMérieux, DIAM, Biolaffort, Fermentis, Nadalié, Lallemand, Rémy-Martin et Cie, Courvoisier, Bellot, Bucher Vaslin, Leco.

Eventually we want to thank the wine and spirits providers, more particularly Conseil Interprofessionnel du Vin de Bordeaux (CIVB, Bordeaux wine Council), Union Grands Crus Classés (Association of classified growths), Crus Classés of Graves, Champagne Bollinger, Château Cos d'Estournel, Château Couhins INRA, Fleur la Mothe, CVBG Dourthe-Kressmann, ODG (organisms controlling denominations of appellation origin) of Lalande de Pomerol, Fronsac, Saint Emilion and Sauternes as well a Union of producers from Saint Emilion, and Hennessy company.

We will do our best to make sure that you will enjoy these days and wish you a very pleasant congress

The EnoIVAS 2019 Organizing Committee

**International Symposium of Oenology of Bordeaux (Oeno)** was created in 1963 by **Pr Jean Ribéreau-Gayon** and **Dr Emile Peynaud** to celebrate the 100th anniversary of Louis Pasteur work on wine. The symposium continued in 1967, 1977, 1989 and then in 1995, 1999, 2003, 2007, 2011, that is each four years, under the lead of **Pr Pascal Ribéreau-Gayon**, then **Pr Yves Glories**, **Pr Aline Lonvaud**, **Pr Bernard Donèche** and celebrated in 2015 its 10th anniversary. **Oeno** symposia are consistent with the principle of general-interest symposia covering the fields of enological science allowing exchanges within a multidisciplinary scientific community dedicated to wine sciences. Thus, 4 traditional themes - Plant environment and grape quality, Grape and wine microorganisms, Wine chemistry, Sensory analysis - are the foundation of the symposia. The research questions have evolved: enological research initially focused on wine clarity and instability problems, then on fermentation control, more recently, the qualitative potential of fruit and its evolution during vinification and aging has been widely studied as well as the parameters of sensory perception.

**In Vino Analytica Scientia (IVAS)** was initiated by the Divisions of Analytical Chemistry and Food Chemistry of the European Association for Chemical and Molecular Sciences, with **Pr Christian Ducauze** and the late **Pr Robert Kellner** as initial advocates. 2 first edition of IVAS were organized in Bordeaux (1977, 2001) then in various places, with a rhythm of congress organization every 2 years (2003 Aveiro, Portugal ; 2005 Montpellier, France ; 2007 Melbourne, Australia ; 2009 Angers, France ; 2011 Graz, Austria ; 2013 Reims, France ; 2015 Trento, Italy ; 2017 Salamanca, Spain). This international meeting provides a unique international platform for interchange between researchers dedicated to the different aspects of the chemistry of wine and spirit production including environmental concerns, vines, grapes, and final products, as well as metabolomics, proteomics, chemical and biochemical reactions, chemometrics, microbiology, and sensory evaluation establishing a forum to discuss and present the latest developments in Analytical Science.

Dear colleagues,

It is a great pleasure for us to welcome you in Bordeaux for this joint scientific meeting, **ENOIVAS 2019**, organized under the patronage of OIV and of European Chemical Society. Oenology in its scientific dimension makes it possible to bring together a large community of researchers from various fields of expertise to share their knowledge and experience around wine and spirits.

Thus, as part of the organization of the 11th International Oenology Symposium, the Oenology Research Unit, Institute of Vine and Wine Sciences Bordeaux-Aquitaine, proposed to the IVAS Scientific Committee to host the 11th edition of the In Vino Analytica Scientia Congress.

As a result, during 4 days, from June 25th to June 28th, 2 international congresses are exceptionally held in the context of a joint organization under the title ENOIVAS 2019. The traditional topics of OENO and IVAS congresses will be considered, with a first day on June 25, built with communications on the following usual themes of OENO (Plant and Environment, Grape quality, Grape and wine microorganisms: diversity and adaptation, Oenological Practices and Process). Then, from June 26th to June 28th, communications on traditional topics of IVAS shall be considered with the following themes : Analysis and composition of grapes, wines, wine spirits, Chemical and Biochemical reactions including grape and wines microorganisms impact, Analytical developments from grape to wine, spirits : omics, chemometrics approaches, Sensory properties including psychophysics, experimental economy, connections with neurosciences. That means that if analytical chemistry and sensory analysis are usually considered in Oeno symposium, the 2019 version of the OENO takes on a more analytical tone and for researchers familiar with IVAS, it integrates more fields of Oenology.

7 speakers have been invited to introduce the different thematic fields of the congress and the evaluation of the papers by the respective scientific committees led to a scientific program of 54 oral communications, as well as 17 short communications at the end of the sessions. 3 workshops have been organized on the fields of grape and wine microorganisms, wine stabilization practices and processes, analytical tools using electromagnetic spectroscopy techniques respectively. Then, 210 poster communications have been selected that is in total close to 310 communications.

We believe that the diversity of these communications will create a favorable context for scientific exchanges, through enabling our international community to contribute to the knowledge on wine as well as to support the wine sector in the face of its challenges.

We wish you an excellent symposium **ENOIVAS 2019** rich in discoveries and fruitful exchanges!

On the behalf of IVAS Scientific Committee  
Dr Veronique Cheynier, Conference President  
Pr. Fulvio Mattivi, President of the Scientific Committee

On the behalf of OENO Scientific Committee  
Pr. Philippe Darriet



# Scientific committee

## IVAS 2019

Véronique CHEYNIER (France) Conference President

Fulvio MATTIVI (Italy) Elected Chair

### Members:

Marco ARLORIO (Italy)

Andrew CLARK (Australia)

Sue EBELER (USA)

Bruno FEDRIZZI (New Zealand)

Teresa ESCRIBANO (Spain)

Erich LEITNER (Austria)

Axel MARCHAL (France)

Chantal MAURY (France)

Doris RAUHUT (Germany)

Jorge RICARDO DA SILVA (Portugal)

Silvia M. ROCHA (Portugal)

Christian ROLANDO (France)

Douglas RUTLEDGE (France)

Dietrich VON BAER (Chile)

Elizabeth WATERS (Australia)

### Invited members for GENOIVAS 2019

Michael JOURDES (France)

Stéphanie MARCHAND-MARION  
(France)

Isabelle MASNEUF-POMAREDE (France)

Tristan RICHARD (France)

Philippe DARRIET (France)

## OENO 2019

Philippe DARRIET (France)

Conference President

### Members:

Warren ALBERTIN (France)

Jean-Christophe BARBE (France)

Marina BELY (France)

Carole CAMARASA (France)

Vittorio CAPOZZI (Italy)

Alain DELOIRE (France)

Laurence GENIS-DENIS (France)

Remy GHIDOSSI (France)

Eric GIRAUD HERAUD (France)

Eric GOMES (France)

Régis GOUGEON (France)

Sabine GUILLAUMIE (France)

Vladimir JIRANEK (Australia)

Patrick LUCAS (France)

Cristina REGUANT (Spain)

Gilles de REVEL (France)

Patricia TAILLANDIER (France)

Pierre-Louis TEISSEDE (France)

Josep VALLS

Pierre WAFFO-TEGUO (France)

Stéphanie WEIDMANN-DESROCHE  
(France)

Tuesday June 25th - DAY 1		Wednesday June 26th DAY 2		Thursday June 27th DAY 3		Friday June 28th DAY 4	
8 h 00	Registration from 8:00 am	9 h 00	Topic Analysis and composition of grapes, wines, wine spirits : invited speaker Pr <b>ANDRE de VILLIERS</b>	09 h 00	Topic Analytical developments from grape to wine, spirits : omics, chemometrics approaches Key note Pr <b>CHIARA CORDERO</b>	09 h 00	Topic Sensory properties, psychophysics, experimental economy, connections with neurosciences Key note Pr <b>HILDEGARDE HEYMANN</b>
9 h 00	OENOIVAS 2019 Conference Opening	9 h 30	Analysis and composition of grapes, wines, wine spirits (6 communications)	09 h30	Topic Analytical developments from grape to wine, spirits : omics, chemometrics approaches (6 communications)	09 h 30	Topic Sensory properties, psychophysics, experimental economy, connections with neurosciences (6 communications)
9 h 30	Topic Plant and Environment Grape Quality (5 communications)	11 h 00	Coffee break	11 h 00	Coffee break	11 h 00	Coffee break
11 h 00	Coffee break	11 h 45	Topic Analysis and composition of grapes, wines, wine spirits (4 communications)	11 h 30	Topic Analytical developments from grape to wine, spirits : omics, chemometrics approaches (4 communications)	11 h 30	Topic Analysis and composition of grapes, wines, wine spirit (3 communications)
11 h 45	Œnological Practices and Process (4 communications)	12 h 45	Lunch time	12 h 45	Lunch time	12 h 15	Conference closure Pr <b>STUART FIRESTEIN</b> then <b>symposium conclusion</b> , announcement next IIVAS
12 h 45	Lunch time	14 h 00	Poster Session	14 h 00	SHORT COM	13 h 15	Lunch Time
14 h 00	Poster Session	15 h 00	Topic Chemical and Biochemical reactions, including grape and wines microorganisms impact (KEY NOTE Dr <b>JOHN DANILEWICZ</b> + 4 communications)	15 h 00	Transportation to Cité du Vin	14 h 30	End of Congress
15 h 00	Topic Grape and Wine microorganisms Key note Pr <b>MATT GODDARD</b> (then 6 communications)	16 h 30	Coffee break	16 h 00	Cité du Vin visit		
17 h 00	Coffee break	17h00	Chemical and Biochemical reactions, including grape and wines microorganisms impact (KEY NOTE Pr <b>DORIS RAUHUT</b> + 4 communications)	19 h 00	Cité du Vin closure		
17 h 30	Œnological Practices and Process (4 communications)	18 h 30	Workshop spectroscopy	19 h 30	Gala dinner and awards		
18 h 30	Workshop Grape and Wine microorganisms						
20 h 00	Welcome cocktail OENOIVAS 2019						

## ENO IVAS 2019 PROGRAMME

### Tuesday June 25<sup>th</sup> DAY 1

08 h 00 – 09 h 00 **Registration**

09 h 00 – 9 h 30 **Conference opening**

09 h 30 – 11 h 00 **Topic 1 : Plant and Environment/ Grape quality**

**Chair : Laurence GENY-DENIS and Chantal MAURY**

**09 h 30 – I.1** Adapting wine production to climate change through the exploration of the diversity of *Vitis vinifera* cultivars. DESTRA C. A. *et al.*, ISVV Univ. Bordeaux

**9 h 45 – I.2** Viticultural zoning of central Chile based on bioclimatic indexes and the impact of climate warming.  
BORDEU E. *et al.*, Pontifical Catholic University of Chile

**10 h 00 – I.3** Mitigating the effects of climate change on berry composition by canopy management  
GUILLAUMIE S. *et al.*, ISVV Univ. Bordeaux

**10 h 15 – I.4** Aroma chemical profiles characterization of wines produced with Moristel grape harvested at different time points  
ARIAS I. *et al.*, Univ. Zaragoza, Univ. Rioja

**10 h 30 – I. short com 1** Influence of pre-harvest elicitors treatment during ripening period on phenolic composition in Monastrell grapes  
GIL-MUÑOZ R. *et al.*, Univ. Sevilla

**10 h 35 – I. short com 2** Coming of age: do old vines actually produce berries with higher enological potential than young vines? A case study on the Riesling cultivar  
GOMES E. *et al.*, ISVV Univ. Bordeaux

**10 h 40 – I. short com 3** Nitrogen status of vines influences aged wines aromas. Examples of aged Champagne reserve wines and red Bordeaux wines  
LE MENN *et al.*, ISVV Univ. Bordeaux

11 h 00 – 11 h 45 **Coffee break – Poster session**

11 h 45 – 12 h 45 **Topic 3 : Oenological Practices and Process**

**Chair : Elisabeth WATERS and Pierre-Louis TEISSEDE**

**11 h 45 – III.1** Grape seed powder as an alternative to bentonite for wine fining  
ROMANINI E. *et al.*, Univ. Sacred Heart, Piacenza.

**12 h 00 – III.2** Foamability of bentonite treated wines: impact of new Acacia gum fractions obtained by Ionic Exchange Chromatography (IEC)  
DOCO T. *et al.*, Univ. Montpellier, Montpellier SupAgro INRA

**12 h 15 – III.3** Optimizing the use of bentonite for better control of haze formation in white and rosé wines  
BACH B. *et al.*, Changins, Univ. Western Switzerland

**12 h 30 - III.4** Simplifying the measurement of different forms of Cu in wines and strategies for efficient removal

KONTOUDAKIS N. et al. Charles Sturt Univ., National Wine Grape Industry Centre, Waga Waga

**12 h 45 - 14 h 00** Lunch time

**14 h 00 - 15 h 00** Poster session

### **In parallel :**

**15 h 00 - 17 h 00** Special session on advanced analytical methodologies (Satellite Event)

**15 h 00 - 17 h 00** Topic 2 : Grape and wine microorganisms

Chair : Cristina REGUANT and Claire LE HENAFF-LE MARREC

**15 h 00 - II. Key note lecture** Describing and harnessing yeast variation

Prof. Matt GODDARD

**15 h 30 - II.1** Fungal resident flora of a new winery: colonization, dynamics and potential persistence capacities.

ABDO H. et al., IUVV Univ. Bourgogne-Franche Comté

**15 h 45 - II.2** Genetic causes of SO<sub>2</sub> consumption in *Saccharomyces cerevisiae*

PELTIER E. et al., ISVV Univ. Bordeaux

**16 h 00 - II.3** Impact of non-*Saccharomyces* in malolactic fermentation of white and red winemaking

BALMASEDA A. et al., Univ. Rovira i Virgili

**16 h 15 - II.4** Mathematical modeling of fermentation kinetics: a tool to better understand interactions between *Torulaspota delbrueckii* and *Saccharomyces cerevisiae* in mixed cultures

TAILLANDIER P. et al., LGC Univ. Toulouse

**16 h 30 - II.5** Nitrogen metabolism in *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* : towards a better understanding of fermentation aroma production

DIVOL B. et al., Univ. Stellenbosch

**16 h 45 - II.6** Use of antisense RNA technology to modulate gene expression in *Oenococcus oeni*

GRANDVALET C. et al., IUVV Univ. Bourgogne-Franche Comté

**17 h 00 - 17 h 30** Coffee break

**17 h 30 - 18 h 30** Topic 3 : Oenological Practices and Process

Chair : Patricia TAILLANDIER and Doris RAUHUT

**17 h 30 - III.5** Bio-protection by one strain of *M. pulcherrima*: Microbiological and chemical impacts in red wines

SIMONIN S. et al., IUVV Univ. Bourgogne-Franche Comté

**17 h 45 - III.6** Grape solids: new advances on the understanding of their role in enological alcoholic fermentation

CASALTA E. et al., INRA Montpellier SupAgro

**18 h 00 - III.7** Characterization and application of silicon carbide (SiC) membranes to oenology

TREVISAN M. et al., ISVV Univ. Bordeaux

18 h 15 – 19h45

**In parallel :**

**Workshop 1 : Grape and wine microorganisms**

Chair : Stephanie WEIDMANN, Patricia TAILLANDIER and Isabelle MASNEUF-POMAREDE

or

**Workshop 2 Œnological Practices and Process : Wine stabilization**

Chair : Jean Christophe BARBE and Remy GHIDOSSI

19h45 Welcome cocktail Bordeaux INP

**ŒNO IVAS 2019 PROGRAMME**

**Wednesday June 26<sup>th</sup> DAY 2**

**9 h 00 - 11 h 00 Topic 4 : Analysis and Composition of Grapes, Wines, wine Spirits**

Chair : Stéphanie MARCHAND-MARION and Teresa ESCRIBANO-BAILON

**9 h 00 - IV Key note Lecture Multidimensional chromatography in grape and wine analysis**  
André de VILLIERS, Univ. Stellenbosch

**9 h 30 - IV.01 A tool for catching mice in wine: development and application of a method for the detection of mousy off-flavour compounds in wine.**  
HAYASAKA Y. *et al.*, The Australian Wine Research Institute

**9 h 45 - IV.02 Trials with machine harvested Sauvignon blanc: the importance of grape transport time and temperature**  
PARISH-VIRTUE K. *et al.*, Univ. Auckland

**10 h 00 - IV.03 HPLC-MS analysis of carotenoids as potential precursors for 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in Riesling grapes**  
GREBNEVA Y. *et al.*, The Australian Wine Research Institute

**10 h 15 - IV.04 The limonene-derived mint aroma compounds in red wines. Recent advances on analytical, chemical aspects and sensory aspects**  
LISANTI M. T. *et al.*, Univ. Napoli

**10 h 30 - IV.05 Evolution of the crown procyanidins during wine making and aging in bottle**  
JOUIN A. *et al.*, ISVV Univ. Bordeaux

**10 h 45 - IV.06 The fundamental role of pH in the anthocyanin chemical behavior and in their extractability during winemaking**  
FORINO M. *et al.*, Univ. Napoli

**11 h 00 -11 h 45 Coffee break – Poster Session**

**Chair : Michael JOURDES and Christian ROLANDO**

**11 h 45 - IV.07** Wine fining with yeast protein extract: effect on polyphenol composition and the related sensorial attributes  
FRANCISCO T. *et al.*, Univ. Porto

**12 h 00 - IV. 08** Beyond colors of rosé wines: Impact of origin and winemaking technology on their color, polyphenol and thiol compositions  
SAUCIER C. *et al.*, INRA Montpellier SupAgro, Univ. Montpellier

**12 h 15 - IV. 09** Changes in red wine composition during bottle aging: Impacts of viticultural conditions and oxygen availability  
ZHANG X. *et al.*, Charles Sturt Univ. National Wine Grape Industry Centre, Waga Waga

**12 h 30- IV. Short com 1** Correlation between skin cell wall composition and phenolic extractability in Cabernet Sauvignon wines  
MEDINA PLAZA C. *et al.*, Univ. California, Davis

**12 h 35- IV. Short com 2** Dispersive Liquid-Liquid Microextraction for the quantification of terpenes in wines  
BERGLER G. *et al.*, INRA Montpellier SupAgro

**12 h 40- IV. Short com 3** Impact of press fractioning on Pinot noir and Pinot meunier grape juice and wine compositions and colour.  
MARCHAL R. *et al.*, Univ. Reims

**12 h 45 - 14 h 00      Lunch time**

**14 h 00 - 15 h 00      Poster session**

**15 h 00 - 17 h 00      Topic 5 : Chemical and Biochemical Reactions including grape and wines microorganisms impact**

**Chair : Andrew CLARK and Tristan RICHARD**

**15 h 00 - V. Key note lecture 1. The role of Fe and Cu in wine oxidation.**  
John DANILEWICZ, England

**15 h 30 - V.1** What is the fate of oxygen consumed by red wine? Main processes and reaction products  
MARRUFO-CURTIDO A. *et al.*, Univ. Zaragoza

**15 h 45 - V.2** Impact of glutathione-rich inactivated yeast on wine chemical diversity  
BAHUT F. *et al.*, IUVV Univ. Bourgogne-Franche Comté

**16 h 00 - V.3** Are dicysteinyl polysulfanes responsible for post-bottling release of hydrogen sulfide?  
BEKKER M. *et al.*, The Australian Wine Research Institute

**16 h 15 - V.4** NMR approach for monitoring the photo-degradation of riboflavin and methionine  
FRACASSETTI D. *et al.*, Univ. Milano

**16 h 30 - 17 h 00      Coffee break**

17 h 00 – 18 h 30    **Topic 5 : Chemical and Biochemical Reactions including grape and wines microorganisms impact**

**Chair : Dietrich VON BAER and Isabelle MASNEUF POMARÈDE**

**17 h 00 –V. Key note lecture 2. Impact of microbial interactions for the development of flavour characteristics in wines**

Doris RAUHUT, Hochschule Geisenheim University

**17 h 30 – V.5 Study of yeast biocatalytic activity on grape aroma compounds**

SLAGHENAUF D. *et al.*, Univ. Verona

**17 h 45 – V.6 Multi-omics Methods to unravel Microbial Diversity in Fermentation of Riesling Wines**

SIREN K. *et al.*, Univ. Copenhagen

**18 h 00 – V.7 Oenological tannins to prevent *Botrytis cinerea* damage: reduction of laccase activity**

VIGNAULT A. *et al.*, Univ. Rovira i Virgili, ISVV Univ. Bordeaux

**18 h 15 – V.8 When organic chemistry contributes to the understanding of metabolism mechanisms**

CAVELIER F. *et al.*, CNRS, Univ. Montpellier

18 h 30 – 19 h 30 **Workshop on Analytical tools using electromagnetic spectroscopy techniques (Infra-Red, Fluorescence, Raman)**

**Chair : Douglas RUTLEDGE**

19 h 30    **Welcome cocktail Bordeaux INP**

## **ENO IVAS 2019 PROGRAMME**

**Thursday June 27<sup>th</sup> DAY 3**

09 h 00 – 11h45    **Topic 6 : Analytical developments from grape to wine, spirits : omics, chemometrics approaches...**

**Chair : Fulvio MATTIVI and Axel MARCHAL**

**9 h 00 – Topic VI. Key note lecture. Characterization of odorant patterns by comprehensive two-dimensional gas chromatography: challenges, strategies and pleasure behind chemistry**

Chiara CORDERO, Univ. Torino

**9h 30 – VI.1 Integrated multiblock data analysis for improved understanding of grape maturity and vineyard site contributions to wine composition and sensory domains**

SCHMIDTKE L. *et al.*, Charles Sturt Univ. National Wine and Grape Industry Center

**9 h 45 – VI.2 Can wine composition predict quality? A metabolomics approach to assessing Pinot noir wine quality as rated by experts.**

SHERMAN E. *et al.*, Plant and Food Research Center, New-Zealand

**10 h 00 – VI.3 D-Wines: Use of LC-MS metabolomic space to discriminate Italian mono-varietal red wines**

ARAPITSAS P. *et al.*, Foundation Edmund Mach, Trento.

**10 h 15 – VI.4** LC-MS based metabolomics and target analysis to study the chemical evolution of wines stored under different redox conditions.

ONTAÑÓN I. *et al.*, Univ. Zaragoza

**10 h 30 – VI.5** Fully Automated Non-targeted GC-MS Data Analysis

VESTNER J. *et al.*, DLR Rheinpfalz, Neustadt

**10 h 45 – VI.6** q-NMR measurements : quantitative analysis of wine composition applied to Bordeaux red wines authenticity control

RICHARD T. *et al.*, ISVV Univ. Bordeaux

**11 h 00 – 11 h 30** Coffee break – Poster Session

**11 h 30 – 12 h 45** Topic VI : Analytical developments from grape to wine, spirits : omics, chemometrics approaches...

Chair : Silvia ROCHA and Bruno FEDRIZZI

**11 h 30 – VI.7** A new graphical interface as a tool to integrate data from GC-MS and UPLC-MS-QTOF: new compounds related with Port Wine Aging

SILVA FERREIRA A.C. *et al.*, Univ. Porto

**11 h 45 – VI.8** Molecular characterization of wines nucleophilic potential by ultra-performance liquid chromatography high resolution mass spectrometry

ROMANET R. *et al.*, IUVV Univ. Bourgogne-Franche Comté

**12 h 00 – VI. 9** Influence of the malolactic fermentation on wine metabolomics or drastic metabolomics changes due to malolactic fermentation

ALEXANDRE H. *et al.*, IUVV Univ. Bourgogne-Franche Comté

**12 h 15– VI. 10** Different strategies for the rapid detection of haze-forming proteins (HFPs)

CETO X. *et al.*, Univ. Barcelona

**12 h 30 – VI. short com 1** Strategies for sample preparation and data handling in GC-MS wine applications

BUICA A. *et al.*, Univ. Stellenbosch

**12 h 35 – VI. short com 2** Development of a LC-FTMS method to quantify natural sweeteners in red wines

FAYAD S. *et al.*, ISVV Univ. Bordeaux

**12 h 40 – VI. short com 3** Development of a New Method to Understand Headspace Aroma Distribution and Explore the Pre-Sensory Level in Perceptive Interactions Involved

in Red Wine Fruity Aroma Expression

CAMELEYRE M. *et al.*, ISVV Univ. Bordeaux

**12 h 45 – 14 h 00** Lunch time

**14 h 00 – 15 h 00** Fresh research session short com : topic 4, topic 6, topic 7

Chair : Véronique CHEYNIER and Philippe DARRIET

**14 h 00 – IV. short com 4** Identification of cis-2-methyl-4-propyl-1,3-oxathiane as a new volatile sulfur compound (VSC) in wine

CHEN L. *et al.*, Univ. Adelaïde

**14 h 05 – IV. short com 5** Comparison of tannin analysis by protein precipitation and normal-phase HPLC  
HENSEN J.P. *et al.*, Univ. Bonn

**14 h 10 – I. short com 4** Tannins : What place for grape seeds  
ROUSSERIE P. *et al.*, ISVV Univ. Bordeaux

**14 h 15 – V. short com 1** Fructose implication in the sotolon formation in fortified wines: preliminary results  
PEREIRA V. *et al.*, Univ. Madeira

**14 h 25 – V. short com 2** Varietal differences between shiraz and cabernet sauvignon wines revealed by yeast metabolism  
ANTALICK G. *et al.*, Univ. Nova Gorica

**14 h 30 – VII. short com 1** Study of the aromatic oxidation markers of Tempranillo long aged wines  
MISLATA A.M. *et al.*, VITEC, Tarragona

**14 h 35 – VII. short com 2** The sensory profile of astringency: application on Sangiovese wines  
RINALDI A. *et al.*, Univ. Napoli

**14 h 40 –VII. short com 3** Crossed approaches to experimental economics and sensory analysis regarding noble rot sweet wines perception  
HUBERT A. *et al.*, ISVV Univ. Bordeaux

**15 h 00 – 16 h 00** Departure Cité du Vin with Tram B (line B)  
arrival Tram station Cité du Vin, 18 train stations)

**16h 00 ... 19 h 00** Cité du Vin visit (closure at 19 h 00)

**19 h 30** Gala dinner La Faïencerie  
(24, rue Faïencerie, 700 from Cité du Vin, 10 min walk)

## ENO IVAS 2019 PROGRAMME

### Friday June 28<sup>th</sup> DAY 4

**9 h 00 – 11 h 00** Topic VII : Sensory properties, psychophysics, experimental economy, connections with neurosciences

**Chair : Chiara CORDERO and Jorge RICARDO DA SILVA**

**9 h 00 – Topic VII. Key note Lecture** Sensory properties of US west coast Pinot noir wines  
Prof Hildegard HEYMANN Univ. Davis, California

**9 h 30 – VII.1** Comparing the effects of vision, smell and taste in red wine quality judgments by experts: Sensory cues, mental imagery and verbal representations as drivers of consensus in the multisensory space  
CAISSIE A. *et al.*, ISVV Univ. Bordeaux

**9 h 45 – VII.2** Does wine expertise influence semantic categorization of wine odors?  
KOENIG L. *et al.*, ESA Angers Univ. Bretagne Loire

**10 h 00 – VII.3** Exploring multisensory interactions through the study of astringency diversity of mono-varietal Italian red wines

PIOMBINO P. *et al.*, Univ. Napoli

**10 h 15 – VII.4** Red wine astringency: Evolution of tribological parameters during different harvest dates

BROSSARD N. *et al.*, Pontifical Catholic University of Chile

**10 h 30 – VII.5** Sensory evaluation of grape berries: predictive power for sensory properties of Sauvignon blanc, Riesling and Pinot Noir wines

FISCHER U. *et al.*, DLR Rheinpfalz Neustadt

**10 h 45 – VII.6** Can varietal 'apricot' aroma of Viognier wine be controlled with clonal selection and harvest timing?

SIEBERT T. *et al.*, The Australian Wine Research Institute

**11 h 00 - 11 h 30** Coffee break

**11 h 30 - 12 h 00** Topic IV : Analysis and Composition of Grapes, Wines, wine Spirits

Chair : André de VILLIERS and Kleopatra CHIRA

**11 h 30 - IV.10** Key odorants responsible for the sensory spaces defining the different aroma potentials of grenache and tempranillo grapes

ALEGRE Y. *et al.*, Univ. Zaragoza

**11 h 45 - IV. 11** Impact of grape maturity on esters content and sensory characters in wines fermented with yeast strains of different genetic backgrounds

BARBE J.C. *et al.*, ISVV Univ. Bordeaux

**12 h 00 - IV. 12** Aroma characterization of aged Cognac spirits: Contribution of volatile terpene compounds

THIBAUD F. *et al.*, ISVV Univ. Bordeaux

**12 h 15** Conference Closure Modulation at the Periphery: what is the nose telling the brain

Stuart FIRESTEIN, Univ. Columbia New York

**12 h 45** Symposium conclusion – Announcement next IVAS

**13 h 15-14 h 30** Lunch time - End of the CONGRESS

## Workshops

### Topic 2 Grape and Wine microorganisms Workshop

- II. WS 1** Influence of mixed fermentations with *Starterella bacillaris* and *Saccharomyces cerevisiae* on malolactic fermentation by *Lactobacillus plantarum* and *Enococcus oeni* in wines  
ENGLEZOS V. *et al.*, Univ. Torino
- II. WS 2** May lactic acid bacteria play an important role in sparkling wine elaboration ?  
DIMOPOULOU M. *et al.*, ISVV Univ. Bordeaux
- II. WS 3** New antibacterial peptides produced by *Saccharomyces cerevisiae* responsible for the inhibition of malolactic fermentation  
NEHME N. *et al.*, Lebanese Univ.
- II. WS 4** Extracellular substances of lactic acid bacteria interests in biotechnological practices applied to enology  
ELICHIRY-ORTIZ P. *et al.*, IUVV Univ. Bourgogne-Franche Comté
- II. WS 5** Application of high-throughput sequencing tools for characterisation of microbial communities during alcoholic fermentation  
TzeMAK S. *et al.*, Univ. Copenhagen
- II. WS 6** Flor yeast diversity and dynamics in biologically aged wines  
DAVID-VAIZANT V. *et al.*, IUVV, Univ. Bourgogne-Franche Comté
- II. WS 7** Yeast diversity in *vitis labrusca* l. Ecosystems  
ROSA A.L. *et al.*, Catholic Univ. Cordoba, Argentina
- II. WS 8** Influence of cell-cell contact on yeast interactions and exometabolome  
PETITGONNET C. *et al.*, IUVV, Univ. Bourgogne-Franche Comté
- II. WS 9** Non-*Saccharomyces* yeast nitrogen consumption and metabolite production during wine fermentation  
BLOEM A. *et al.*, Univ. Montpellier, INRA Montpellier SupAgro
- II. WS 10** Population-wide diversity study in *Lachancea thermotolerans* highlights superior starters for winemaking  
HRANILOVIC A. *et al.*, Univ. Adelaide
- II. WS 11** Bioprotective non-*Saccharomyces* yeast as an alternative to sulfites for the winemaking process  
WINDHOLTZ S. *et al.*, ISVV Univ. Bordeaux

### Topic 3 Oenological Practices and Processes Workshop on wine stabilization

- III. WS 1** Colloidal stabilization of young red wine by Acacia senegal gum: the major implication of protein-rich arabinogalactan-proteins  
NIGEN M. *et al.*, Montpellier SupAgro

**III.WS 2** Evaluating alternatives to cold stabilization in wineries : the use of carboxymethyl cellulose, potassium polyaspartate, electro dialysis and ion exchange resins and the results after one year in the bottle  
GÓMEZ PLAZA E. *et al.*, Univ. Murcia

**III. WS 3** Research on the origin and the side effects of chitosan stabilizing properties in wine  
DOLS-LAFARGUE M. *et al.*, ISVV Univ. Bordeaux

**III. WS 4** The affinity of white wine proteins for bentonite is dependent on wine composition and is directly related to their thermal stability / sensitivity  
PONCET-LEGRAND C. *et al.*, Univ. Montpellier, INRA Montpellier SupAgro

**III. WS 5** Importance of matrix effects (wine composition) on protein stability tests of white and rosé wines  
MEISTERMANN E. *et al.*, IFV Colmar

**III. WS 6** Removal of white wine heat unstable proteins by using proteases and flash pasteurization. Comparison with bentonites treatments  
MARCHAL R. *et al.*, Univ. Reims Champagne-Ardenne

#### Topic 4 and 6

#### Workshop on Analytical tools using electromagnetic spectroscopy techniques (Infra-Red IRTF, Fluorescence, Raman)

**IV. WS 1** Determination of titratable acidity, sugar and organic acid content in red and white wine grape cultivars during ripening by VIS-NIR hyperspectral imaging  
GABRIELLI M. *et al.*, ESA Angers

**VI. WS 2** Use of Fourier Transform Infrared Spectroscopy (FT-IR) to rapidly verify the botanical authenticity of gum arabic  
MALACARNE M. *et al.*, Foundation Edmund Mach, Trento

**VI. WS 3** Monitoring small-scale alcoholic fermentations using a portable FTIR-ATR spectrometer and multivariate analysis  
CAVAGLIA J. *et al.*, Univ. Tarragona

**VI. WS 4** Development of FTIR partial least squares models for polyphenol quantification in red wine during fermentation  
MIRAMONT C. *et al.*, ISVV Univ. Bordeaux

**IV. WS 5** multivariate characterization of italian monovarietal red wines using FTIR spectroscopy  
VERSARI A. *et al.*, Univ. Bologna

**IV. WS 6** CIEDE 2000 colour difference value as a parameter for tracing the ageing process on wood aged spirits  
DELGADO GONZÁLEZ M.J. *et al.*, Univ. Cadiz

**IV. WS 7** Monitoring gas-phase CO<sub>2</sub> in the headspace of champagne glasses through diode laser spectrometry  
MORIAUX A.L. *et al.*, Univ. Reims Champagne-Ardenne

**VI. WS 8** Discrimination of white wines by RAMAN spectroscopy coupled with chemometric methods  
MAURY C. *et al.*, ESA Angers

# List of posters

## Topic I

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Elisa MARGUERIT, [Loïc Lagalle](#), Maria Lafargue, Jean-Pascal Tandonnet, Jean-Pascal Goutouly, Isabelle Becavin, Maryline Roques, Laurent Audeguin, Nathalie Ollat

#### **I.P.2 LEAF METABOLOMIC CHANGES INDUCED IN A COMMON SCION ON DIFFERENT ROOT-SYSTEMS AND UNDER DIFFERENT IRRIGATION REGIMES**

Misha KWASNIEWSKI, Mani Awale, Connie Liu

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Cédric SAUCIER, Marie ZERBIB, Guillaume CAZALS, Marie-Agnès DUCASSE, Jean-Paul MAZAURIC, Emmanuelle MEUDEC, Christinele GUERNEVE, Alexander LEPAK, Bernd NIDETSKY, Véronique CHEYNIER, Nancy TERRIER, Christine ENJALBAL

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ROCIO GIL-MUNOZ, Diego F.P aladines-Quezada, Juan D. Moreno-Olivares, José I. Fernández-Fernández, Raquel Gonzalez, Carlos Moro, Alberto Guadarrama

#### **I.P.5 USING WINE QUALITY INDICATORS TO EVALUATE LIFE CYCLE ENVIRONMENTAL IMPACTS OF WINE: ROOTSTOCK & IRRIGATION FIELD STUDY MEETS LIFE CYCLE ASSESSMENT.**

Christine COSTELLO, Pouneh Abbasian, Misha T. Kwasniewski, Zoë Migicovsky

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Ulrich FISCHER, Michael Ziegler

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Mani AWALE, Connie Liu, Misha Kwasniewski

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Toni EL KHAWAND, Julien Gabaston, David Taillis, Marie-Laure Iglesias, Eric Pedrot, Josep Valls-Fonayet, Jean-Michel, Mérillon, Alain Decendit, Stéphanie Cluzet, Tristan RICHARD

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David TAILLIS, Oussama Becissa, Julien Gabaston, Tristan Richard, Jean-Michel Mérillon, Stéphanie Cluzet

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M<sup>a</sup> Carmen GARCIA-PARRILLA, Edwin Fernandez-Cruz, Ana B Cerezo, Emma Cantos, Tristán Richard, Ana M. Troncoso

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Stéphanie CLUZET, Aleksandra Burdziej, Cezary Pączkowski, Agnès Destrac-Irvine, Tristan Richard, Anna Szakiel, Stéphanie Cluzet

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Emilie BRUEZ, Céline Cholet, Cécile Thibon, Pascaline Redon, Soizic Lacampagne, Tommaso Martignon, Massimo Guidici, Philippe Darriet, Laurence Geny

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Astrid BUICA, Valeria Panzeri, Hilaria Ipinge

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Ignacio GARCÍA-ESTÉVEZ, Rebeca Ferreras-Charro, Elvira Manjón, M. Teresa Escribano-Bailón

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Marie ANDRE, Sandra ISMAIL, Audrey BARSACQ, Laurence MERCIER, Laurence GENY-DENIS

## Topic II

### Grape and wine microorganisms : diversity and adaptation

**II.P. 1 *BRETTANOMYCES BRUXELLENSIS*, A HIGHLY GENETICALLY DIVERSE SPECIES:**

**A FOCUS ON WINE STRAINS**

Alice CIBRARIO, Marta Avramova, Patricia Ballestra, Warren Albertin, Isabelle Masneuf-Pomarède, Marguerite Dols-Lafargue

**II.P. 2 INVESTIGATION OF THE CAPACITY OF *BRETTANOMYCES BRUXELLENSIS* STRAINS TO FORM BIOFILM**

Manon LEBLEUX, Hany Abdo, Warren Albertin, Julie Maupeu, Julie Laurent, Hervé Alexandre, Michele Guiloux-Benatier, Stéphanie Weidmann, Sandrine Rousseaux

**II.P. 3 VITIS SPECIES DO NOT DRIVE POPULATION STRUCTURE IN *CANDIDA ZEMPLININA* (SYNONYM *STARMERELLA BACILLARIS*) SPECIES**

Warren ALBERTIN, Alberto Luis Rosa, Maria Laura Raymond Eder, Francisco Conti, Marina Bely, Isabelle Masneuf-Pomarede

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Sarah PLOYON, Jérôme Vialaret, Anne-Sophie Walker, Christophe Hirtz, Cédric Saucier

**II.P. 5 GENETIC DISTRIBUTION OF *BRETTANOMYCES BRUXELLENSIS* STRAINS ISOLATED FROM GREEK WINE**

Maria DIMOPOULOU, Isabelle MASNEUF-POMAREDE, Warren ALBERTIN

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Frédérique JULLIAT, Stéphane Guyot, Hervé Alexandre, Cosette Grandvalet

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Marguerite DOLS-LAFARGUE, Alice Cibrario, Hany Abdo, Margot Paulin, Cécile Miot-Sertier, Patricia Bal-lestra, Warren Albertin-Leguay, Isabelle Masneuf-Pomarede

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Raffaele GUZZON, Sergio Moser, Tiziana Nardin, Tomas Roman, Loris Tonidandel, Roberto Larcher

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Julie MAUPEU, Amélie VALLET-COURBIN, Lucie DUTILH, Marine LUCAS, Patrick LUCAS, Isabelle MASNEUF-POMAREDE

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Sílvia M.ROCHA, Carina Costa, Denisa Mateus, Susana Sousa, Frank S. S. Rogerson, Cláudia Coimbra, João S. Simões

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Vincent RENOUF, Laetitia Etourneau, Cécile Arfeuillere

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M. Rosario SALINAS, Víctor M. Tolosa, M. Luisa Lerma, Pilar Castillo, M. Rosario Salinas, Ramona M. Muñoz

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Nadine FEGHALI, Warren Albertin, Edward Tabet, Giacomo Zara, Marilena Budroni, Isabelle Masneuf-Po-marede

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Patricia BALLESTRA, Angela Ferreres Cabanes, Audrey Robidart, Béatrix Braneyre, Jade Bosviel, Cécile Miot-Sertier, Alice Cibrario, Warren Albertin, Isabelle Masneuf Pomarede, Marguerite Dols-Lafargue

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Geraldine KLEIN, Camille Eicher, Cosette Grandvalet, Pascale Winckler, Jean-Luc Parrou, Hervé Alexandre

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Elissa ABI-HABIB, Aude Vernhet, Stéphanie Roi, Stephanie Carrillo, Céline Poncet-Legrand

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Encarna GÓMEZ-PLAZA, Andrea Osete-Alcaraz, Ana Belén Bautista-Ortín

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Florian BAHUT, Youzhong Liu, Rémy Romanet, Nathalie Sieczkowski, Hervé Alexandre, Philippe Schmitt-Kopplin, Maria Nikolantonaki, Régis D. Gougeon

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Rémy JUNQUA, Liming Zeng, Rémy Ghidossi, Alexandre Pons

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Pascal POUPAULT<sup>2</sup>, Fabrice DOUCET<sup>3</sup>

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Mariona GIL I CORTIELLA, Cristina Úbeda, Jose Ignacio Covarrubias, Alvaro Peña-Neira

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Allie KULCSAR, Anais Faucon, Paul Smith, Marlize Bekker

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JORGE M. RICARDO-DA-SILVA, Inês Nunes, Ana C. Correia, António M. Jordão

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Tiziana NARDIN, Tomas Roman Villegas, Giorgio Nicolini, Mario Malacarne, Raffaele Guzzon, Roberto Larcher

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Remedios CASTRO, Lorena Díaz-Guzmán, Carmelo García-Barroso, Enrique Durán-Guerrero

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Enrique DURÁN-GUERRERO, Lorena Díaz-Guzmán, Carmelo García-Barroso, Remedios Castro

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Luigi Picariello, ANGELITA GAMBUTI

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Alessandra FERRANDINO, Adalberto Merighi, Vittorino Novello, Laura Lossi

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Stéphanie MARCHAND, Gilles de Revel

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Nicolas LE MENN, Richard Marchal, Dominique Demarville, Sophie Tempère, Gilles de Revel, Stéphanie Marchand







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# Lectures

## DESCRIBING AND HARNESSING YEAST VARIATION

Pr. Matthew Robert GODDARD

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*Saccharomycetaceae* yeasts are seemingly ubiquitous in terms of habitat and geography and encapsulate significant metabolic diversity. The role of these organisms in 'natural' ecosystems is not well described. However, when associated with the processing of food and beverages the value of the fermentative and metabolic actions of many members of this Family is much clearer.

This talk will provide an overview of the community ecology and distribution of *Saccharomycetaceae* yeast associated with fruit cultivation, and the conversion of fruits into wine, and attempt to define some of their ecological roles and link this to the value of fermented products. The talk will go onto describe the components of these communities and start to understand how various species interact, and lastly look at novel non-GM tools to create novel strains for the fermentation industry.

## MULTIDIMENSIONAL CHROMATOGRAPHY IN GRAPE AND WINE ANALYSIS

Pr André de VILLIERS

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**Keywords:** Comprehensive two-dimensional gas chromatography, comprehensive two-dimensional liquid chromatography, Mass spectrometry, multidimensional chromatography.

The growing demand for accurate analytical data in support of grape and wine metabolomics research has renewed interest in the development and application of more powerful analytical techniques and instrumentation in this field. In this context, multidimensional chromatographic methods, especially in combination with mass spectrometry (MS), offer several advantages compared to conventional methods for grape and wine analysis.

A brief overview of the fundamental and instrumental aspects of multidimensional gas- and liquid chromatographic separations will be presented, with the emphasis on potential the benefits and unique instrumental considerations for comprehensive two-dimensional gas chromatography (GC×GC) and liquid chromatography (LC×LC). This will serve as background to the discussion of selected applications of GC×GC and LC×LC to grape and wine analysis.

Examples of the GC×GC analysis of wine volatiles will focus on the higher resolving power of the technique, especially suited for the detailed qualitative analysis of a wide range of aroma compounds in a single analysis. The importance of sample preparation methods used in combination with GC×GC-high speed time-of-flight (TOF)-MS analysis will be addressed, as will methods for data analysis. Furthermore, recent work on the hyphenation of GC×GC with high resolution TOF-MS detection will serve to illustrate the potential of this approach for wine analysis.

In terms of LC×LC, the analysis of grape and wine phenolic compounds by on-line hydrophilic interaction chromatography × reversed phase LC (HILIC×RP-LC) hyphenated to diode array, fluorescence and high resolution MS will be used to demonstrate the utility of this emerging technology. Real-life examples of the performance offered by LC×LC for the analysis of anthocyanins, condensed tannins and non-coloured phenolics will be presented. Finally, some recent work on the comprehensive combination of LC×LC separation with ion mobility spectrometry (IMS) and high resolution MS for the improved characterisation of grape and wine phenolics will point the way to future developments in the field.

## THE ROLE OF FE AND CU IN WINE OXIDATION

Dr John DANILEWICZ

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**Keywords:** metals, redox phenomena, oxygen, sulfur compounds

The management of wine oxidation to improve quality and to avoid spoilage is a vital aspect of wine production. Understanding the mechanism of oxidation and the participation of sulfite is therefore important. Oxygen cannot react with wine constituents directly. It accepts electrons singly by reacting with Fe(II), which is accelerated by Cu.  $H_2O_2$  and Fe(III) are produced. Fe(III) oxidizes polyphenols and so redox-cycles. The reduction potential of the Fe-redox couple is determined by its main ligands, tartaric and malic acids. Oxygen is readily reduced by Fe(II) but Fe(III) is not a strong enough oxidant to oxidize catechols, unless the reaction is facilitated by nucleophiles, such as sulfite, which react with the quinones that are produced. Sulfite may add to quinones or reduce them back to catechols. By reacting with  $H_2O_2$  sulfite also prevents the oxidation mainly of ethanol. Consequently, when adequate sulfite is present, the  $SO_2: O_2$  molar reaction ratio should be 2:1, which is so, provided the reaction is followed for adequate time, as the rate of  $O_2$  and  $SO_2$  reaction can differ. This difference is proposed to be due to the initial formation of unstable quinone-sulfite adducts, which decompose slowly.

The Fe(II):Fe(III) ratio decreases as wine is oxidized and this ratio may provide a measure of redox state, as it depends on the relative rate of Fe(III) reduction and Fe(II) oxidation. At  $O_2$  saturation this ratio stabilizes at different levels for different wines but the reduction potential of the Fe-couples do not correspond to potentials that would be measured for wines at that level of  $O_2$  exposure. This finding together with the observation that polyphenols do not exist in equilibrium with their oxidation products cast doubt of the ability of wine so-called reduction potentials to indicate redox state. Red wines in closed containers can react with  $O_2$  sufficiently rapidly so as to maintain very low concentrations despite some  $O_2$  ingress. Low reduction potentials would result, misleadingly indicating reductive conditions. However, Fe(II):Fe(III) ratios show that such wines are oxidizing.

When  $H_2S$  is oxidized, thiols such as cysteine are also present and together these rapidly reduce Cu(II) to Cu(I). The resulting Cu(I) complexes are proposed to aggregate with the production of non-volatile disulfides and polysulfanes.  $O_2$  or Fe(III) oxidize Cu(I) back to the cupric state allowing Cu to redox-cycle. Polysulfanes may be a latent source of  $H_2S$ .

## IMPACT OF MICROBIAL INTERACTIONS FOR THE DEVELOPMENT OF FLAVOUR CHARACTERISTICS IN WINES

Pr. Dr. Doris RAUHUT

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**Keywords:** Wine flavour, interaction of microorganisms, yeasts, lactic acid bacteria, fungi, co-fermentation, aroma compounds

The creation of certain wine flavour characteristics and styles is getting more and more important to meet consumer expectations, to intensify typicality of wines and to guarantee high wine quality. The impact of interactions between microorganisms associated with wine fermentations have been of high interest during the last two decades. In particular, their contribution to flavour complexity and intensification of specific aroma attributes in wines is being investigated by numerous research groups.

One of the most important areas of research is the co-fermentation of selected non-conventional yeasts and strains of *Saccharomyces cerevisiae* to enhance the release and formation of high aroma-active compounds, in particular the extremely odoriferous thiols involved in the varietal aromas of wines due to complementary metabolic reactions of different yeast species and strains. In addition, the attention is related to the increase of monoterpenoids (C<sub>10</sub> compounds) regarded as key compounds in aromatic grape varieties of *Vitis vinifera*.

Furthermore, numerous research studies have demonstrated that lactic acid bacteria (LAB) during malolactic fermentation can enhance the fruity character in white and red wines thanks to the higher potential of enzymes for the production of wine aromas by specific LAB species and strains and due to interaction with certain strains of *S. cerevisiae* and others.

Additionally, there is evidence given by several research studies that some of the key-aroma compounds of botrytized wines could be probably intensified by the interaction of specific yeast strains during fermentation.

The presentation will give a short overview on the impact of microbial interactions for the development of flavour characteristics in wine on the basis of examples from literature references. Novel insights on interactions between yeasts, bacteria and fungi to intensify desired aromas in wines will be pointed out. Future perspectives and challenges with the application of mixed starter cultures from different microorganisms during wine fermentations will be highlighted.

## CHARACTERIZATION OF ODORANT PATTERNS BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY: CHALLENGES, STRATEGIES AND PLEASURE BEHIND CHEMISTRY

Pr. Chiara CORDERO and Pr. Carlo BICCHI

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**keywords:** volatile compounds, comprehensive two dimensional gas chromatography, quantitation

Investigation strategies capable of comprehensively mapping the set of all potential ligands (e.g., sensome-tabolome) that trigger the multimodal perception of food flavor should cut across chemistry and biology and require dedicated analytical tools for an effective and reliable capture of complex patterns of volatiles.

This key-note lecture critically discusses state-of-the-art research in the field of odorants and volatiles characterization in food and wines by comprehensive two-dimensional gas chromatography, illustrating how hyphenation with mass spectrometry and olfactometry, accurate quantitation, suitable sample preparation, and dedicated data mining can capture essential information on odor patterns exploiting the higher level of information on sample sensory features.

## SENSORY PROPERTIES OF US WEST COAST PINOT NOIR WINES

**Pr. Hildegard HEYMANN**

Ron Runnebaum, Annegret Cantu, Alex Yeh, Shelby Byer, Isadora Frias, Travis Witte, Pauline Lestringent

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**Keywords** : Sensory Descriptive analysis, Pinot noir, Volatiles, Non-volatiles

The overall objective of this study is to investigate the sensory differences among Pinot noir wines derived from a single clone (667), and grown on different vineyard locations in the US West Coast over two years (2015 and 2016). The rootstock for the California vineyards is 101-14 and for the two Oregon vineyards in the Willamette Valley the rootstock is Riparia Gloire. The vineyards are located in the following American Vineyard Areas (AVAs): Santa Rita Hills (1), Santa Maria Valley (2), Arroyo Seco (2), Sonoma Coast (2), Russian River Valley (1), Carneros (1), Anderson Valley and the Willamette Valley (2). The vineyard soil, and climatic conditions were monitored. All grapes were harvested at about the same sugar content and transported to Davis where all wines were made in the UC Davis Experimental Winery using the same protocol. The wines from the 2015 and 2016 vintages were evaluated 20 months after harvest in May 2016 and May 2017, respectively. Trained sensory descriptive panels evaluated the wines in triplicate and at the same time the same bottles were used for volatile and non-volatile analyses.

The results indicate that for some of the AVAs the wines from 2015 and 2016 were very similar both sensorially and also chemically, for example both vineyards from Arroyo Seco, whereas for other AVAs the wines differed across the two vintages, for example the Russian River Valley AVA. Additionally, there seems to be a somewhat consistent south to north variation in the wine sensory properties (the Santa Rita Hills AVA is 1400 miles south of the Willamette Valley AVA). However, there are also some within AVA variations that can be explained by soil type, altitude and climate.

## MODULATION AT THE PERIPHERY: WHAT IS THE NOSE TELLING THE BRAIN?

Pr. Stuart FIRESTEIN

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**Keyword:** neurophysiology, sensory neurons, olfactory stimuli, complex odors perception

Modulation of the stimulus is a common property of sensory systems, including both inhibition and enhancement of selected inputs. In all known cases this modulation occurs at higher levels of processing, at synapses of relay neurons between the peripheral sensors and cortical brain regions. Similar modulation has been demonstrated in the flavor (olfactory and taste) system in humans in psychophysical experiments, and are assumed to be due to higher level processing.

Utilizing a new technique in microscopy we have been able to monitor the activity of thousands of individual sensory neurons in the living nasal epithelium of genetically altered mice. When we apply mixtures of odors we find that a given odor species can act to activate, inhibit or enhance the response of other sensory neurons. That is, modulation of the olfactory stimulus appears to occur in the peripheral sensory cells, prior to any additional processing at higher centers.

When we test for similar effects in humans using psychophysical techniques we find similar interactions between odors in a blend. This sets the olfactory system apart from other sensory systems and raises critical new issues as to how the nose and brain communicate to perceive a complex odor world.

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# Oral communications

Following the order of the  
scientific program



# Plant and Environment, Grape quality

## I.O.1

# ADAPTING WINE PRODUCTION TO CLIMATE CHANGE THROUGH THE EXPLORATION OF THE DIVERSITY OF *VITIS VINIFERA* CULTIVARS

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**Keywords:** climat change, phenology, wine, Bordeaux

Major factors involved in wine quality and typicity are soil type, climatic conditions, plant material (rootstock and cultivar), vineyard management practices and winemaking conditions. All these factors interact and growers optimize the output in terms of yield and quality by adapting plant material and management practices to environmental factors (soil and climate). Hence, plant material is region specific, because growers have selected the optimum rootstocks and varieties for their soils and climatic conditions through a long process of trial and error. Climatic conditions have always changed from year to year (the so-called vintage effect), but since three decades a long term trend is observed in most winegrowing regions towards increased temperatures and summer drought. This evolution raises the question whether region-specific plant material (in particular cultivars) will still be optimum in a warmer and dryer climate. To anticipate potential need for cultivar changes in the Bordeaux area, a trial encompassing 52 cultivars called "VitAdapt" was planted in 2009. Beside all references currently used in Bordeaux, the focus was laid on later ripening cultivars which are currently used in warmer regions. Every cultivar is planted with 5 replicates to take into account possible variations in soil composition. Phenology and grape composition from veraison to ripeness was monitored since 2012 and wines were made by micro vinifications in 2016 and 2017 for 20 cultivars. Wines were tasted by a panel of wine professionals familiar with Bordeaux wines and wines were scored for their typicity in relation to what can be expected for Bordeaux wine. Major varietal aroma compounds were analyzed in the wines.

Cultivars varied widely with regard to their precocity. The delay between the most early and latest cultivar is on average 28 days for bud break, 15 days for flowering and 39 days for veraison. A model called Grapevine Flowering Veraison (GFV) was developed and validated on the VitAdapt trial to predict the occurrence of these phenological stages from temperature data. Unsurprisingly, Bordeaux cultivars (and in particular Cabernet-Sauvignon) scored well with regard to Bordeaux wine typicity. Among non-Bordeaux cultivars which showed similar typicity, most were late ripening and had similar phenology, or later phenology, compared to the traditional Bordeaux cultivars. The analysis of key aroma compounds should allow to have a better understanding of the molecular basis of Bordeaux wine typicity and to group cultivars according to their aroma profile. This research will help Bordeaux wine growers to identify cultivars which can potentially be introduced in the Bordeaux cultivar-mix and thus provide a tool to continue to make highly quality, true-to-the-type Bordeaux wines in a changing climate.

## I.O.2

## VITICULTURAL ZONING OF CENTRAL CHILE BASED ON BIOCLIMATIC INDEXES AND THE IMPACT OF CLIMATE WARMING

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**Keywords:** Global warming, bioclimatic indexes, wine quality, aptitude zoning

Climate is considered one of the main factors that determines the aptitude of a specific location for growing grapes and producing high quality wine, being in that sense one of the main elements defining the concept of terroir. Several bioclimatic indexes have been proposed that attempt to describe the climatic aptitude for high quality wine production. Based on this, a study was developed to characterize present and future viticultural potential of Chilean zones considering the dynamic of climate change.

Maps of central Chile were built based in different climatic parameters and the calculation of bioclimatic indexes like Winkler, Huglin, Mean January Temperature, Cold Nights index and Fregoni. Interactive maps obtained allow determining the aptitude of a locality by introducing the geographic coordinates.

In a second step, the future evolution of these climatic parameters was studied considering different models of climatic change (CSIRO-Mk3-6-0; GFDL-CM3 and HadCM3), different scenarios (moderate or severe) and different periods of time. Results obtained are also represented in interactive maps allowing seeing the evolution of a climatic parameter in time depending on the model and severity chosen.

Results obtained show a clear evolution of the aptitude of the main viticultural areas with with an increase in aptitude of regions in the south of Chile that presently have limitations for a good maturation, in particular of red varieties. More to the south and closer to the Pacific Ocean areas that are not suitable for wine production now become apt for wine production Northern areas are in general negatively affected by becoming too hot and having increasing problems with water supply.

### I.O.3

## MITIGATING THE EFFECTS OF CLIMATE CHANGE ON BERRY COMPOSITION BY CANOPY MANAGEMENT

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**Keywords:** climate change, leaf/fruit ratio, berry composition, wine

Primary and secondary metabolites are major components of grape composition and their balances define wine typicality. Global climate change is modifying vine physiology and especially the composition of grape berries at harvest, by decoupling phenolic and aromatic maturities (depending on secondary metabolites) from technical maturity (depending on primary metabolites). These modifications can be limited through vineyard management. One of the rapid and efficient ways to mitigate the effects of climate change is to modify vine canopy, thus modifying the relationships between source and sink.

To face this challenge, we used *Vitis vinifera* cv. Cabernet Sauvignon plants 1) to analyse the response of yield and biochemical composition in ripening berries, including sugars, organic acids, amino acids, phenolic compounds (anthocyanins, flavonols) and aroma molecular makers including methoxypyrazines associated with the green character (low ripening), volatile thiols (and their precursors), as well as furanones and lactones linked with the cooked/dried fruit aromas (overripening), with UHPLC, GC-MS and LC-MS analyses; 2) to link the changes in berry composition with wine quality by microvinification sensory analysis; 3) to study the response of berry transcriptome to canopy manipulation, by RNAseq or qPCR analyses.

The results showed that metabolites had different sensitivities to the modulation of leaf-to-fruit ratios, demonstrating that it is possible to determine an optimal leaf/fruit ratio to reduce sugar concentration in the berry without much impact on the typicality of Bordeaux wines.

**Acknowledgments:** We thank the CIVB for financial support to the CANOGRAPPE project N ° 44233 and France AgriMer for financial support to CANABA project N°414.

## I.O.4

**AROMA CHEMICAL PROFILES CHARACTERIZATION OF WINES PRODUCED WITH MORISTEL GRAPES HARVESTED AT DIFFERENT TIME POINTS****Ignacio ARIAS**

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**Keywords:** Wine aroma, maturity, acetaldehyde, reduction factors

The wine aroma is constituted by hundred of volatile chemical compounds that depend on many viticultural and oenological factors. One of the most important factors that will unequivocally affect the final wine properties is the grape maturity level. Grape ripening is an extremely complex process, in which the metabolites and precursors concentrations change significantly with time. However, the knowledge of how grape ripening affects wine aroma composition is still quite limited. Nowadays, wineries measure parameters such as sugar, pH, acidity and colorimetric tests to evaluate the degree of maturity of the vintage and decide the harvest date, but these analysis do not take into consideration the aromatic potential of the grape.

The objective of the present work is to understand the differences in the aroma chemical profile of Moristel wines from different vineyards harvested at different time points. So, three different vineyards of Moristel grape variety in D.O. Somontano were selected, in two consecutive vintages: two in 2016 and one in 2017. Each block was harvested at different time points followed by microvinifications applying the same fermentation protocol. All of them have been elaborated in triplicated. This was assessed by the analysis of major aroma compounds (GC-FID), trace aroma compounds (GC-MS), methoxypyrazines (TD-GC×GC-MS), polyfunctionalmercaptans (SPE GC-MS), volatile sulfur compounds (BR-VSCs) and total acetaldehyde (HPLC-UV/VIS).

The most important result is that the grapes harvested at 42 days postveraison, that is the "green" ones, produce wines with high concentration of acetaldehyde and low IPT. Hence, low concentration of polyphenols facilitate the accumulation of this compound. Another reason for these acetaldehyde high concentrations could be problems associated with the lack of reduction factors (NADH or NADPH).

This fact is also corroborated with the decreases of branched acid / fusel alcohol and branched ester/fusel alcohol ratios during the maturity. These facts can have very important sensory repercussion, the acetaldehyde and fusel alcohol are components of aroma buffer. Finally, the evolution of certain maturity markers (c-3-hexenol,  $\gamma$ -nonalactona, rotundone) has been also observed, but these target compounds, by themselves, do not seem to have great sensory relevance in the final wines.

This study has help to elucidate how grape maturity state contributes to final Moristel wine aroma profile and possible self-life.

**Acknowledgements** This work has been funded by the Spanish MINECO (Project AGL2014-59840, RTC-2015-3379 and RTC-2016-4935-2) and partly co-funded by the European Union (FEDER). I.A. has received a grant from the Spanish FPU programs. Funding from D.G.A. (T53) and Fondo Social Europeo is acknowledged.



# Grape and wine microorganisms : diversity and adaptation

## II.O.1

# FUNGAL RESIDENT FLORA OF A NEW WINERY: COLONIZATION, DYNAMICS AND POTENTIAL PERSISTENCE CAPACITIES

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**Keywords:** Fungal diversity and dynamics, New winery, Fungal resident flora, *Saccharomyces cerevisiae*

Through the years, extensive studies have been conducted on fungal biodiversity during the winemaking process: from the vineyard until aging. More recently, and as previously described for *Saccharomyces cerevisiae*, the persistence of winery resident flora (non-*Saccharomyces* yeasts) over time and its contribution to the alcoholic fermentation have been demonstrated. Also, winery surfaces were described as a true ecological niche and a transitional habitat for this resident flora.

To our knowledge, no study has been done on the evolution and persistence of indigenous yeast flora in a new winery nor on the capacities of this indigenous flora to persist in the winery environment. Thus, the first objective of this work is to study the diversity and to follow the evolution of fungal communities in a new established winery operating only with indigenous yeast flora and for a period of 3 vintages (2016, 2017 and 2018). For this purpose, samples were collected from three different winery surfaces (soil, walls and equipment), at separate time points (before grape harvest, during the fermenting phase and 3 months after the fermentations) and were analyzed using the Mi-Seq sequencing. In a second objective, genetic diversity, persistence in winery environments and the implantation in must /or wine of indigenous *S. cerevisiae* isolates were monitored for all the collected samples using microsatellites PCRs. In addition, the killer character and biofilm formation of different isolated strains were investigated to determine potential capacities to persist in winery environments.

The results obtained showed a high fungal diversity (yeasts, fungi and mold) on the 3 winery environments even before the first grape harvest (2016). As for yeasts, previously described genera (*Candida*, *Metschnikowia*, *Rhodotorula*, *Saccharomyces*, *Wickerhamomyces*, ...) have been identified on winery surfaces but also yeast genera (*Buckleyzyma*, *Curvibasidium*, *Leucosporidium*, ...) that have not been before described in the winemaking process. Then, the observed fungal diversity showed evolution over time and dependently according to each of the studied environment. Additionally, some fungal equilibria appears to take place between genera such as *Aureobasidium*, *Candida* and *Wickerhamomyces*.

Concerning indigenous *S. cerevisiae* strains, our results demonstrated the potential implantation and persistence of some strains present in the winery environment during 2017 and 2018 vintages and during the alcoholic fermentations. Thus, selected strains of indigenous *S. cerevisiae* seem to have different physiological characteristics that could explain their potential persistence in winery environments.

## II.O.2

GENETIC CAUSES OF SO<sub>2</sub> CONSUMPTION IN *SACCHAROMYCES CEREVISIAE*Emilien PELTIER (1,2)

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**Keywords:** Yeast, QTL mapping, SO<sub>2</sub>

SO<sub>2</sub> is used during winemaking for its anti-oxidative and anti-microbial properties. A high SO<sub>2</sub> concentration in the wine has negative impacts by hiding wine aromas and delaying malolactic fermentation. SO<sub>2</sub> concentration is also a source of health concerns and is therefore legally regulated. During the alcoholic fermentation SO<sub>2</sub> can be produced or consumed by the yeast *Saccharomyces cerevisiae* with a high variability depending on the strain that accomplish the fermentation. The selection of industrial strains leaving less SO<sub>2</sub> at the end of fermentation is therefore of great interest.

In this study we implemented a QTL (Quantitative Trait Loci) mapping program to identify genetic factors that impact SO<sub>2</sub> production by yeast during fermentation. This approach requires the study of a large progeny in segregation that must be characterized genetically and phenotypically. The establishment of a statistical link between genotype and phenotype allows the localization of QTLs that have an impact on the characters.

Small-scale fermentations in 10 ml screw cap vessels coupled with robotized enzymatic allowed us to measure SO<sub>2</sub> profile of several hundred individuals from two progenies. These two progenies were also genotyped by whole genome sequencing providing saturated genetic maps of thousands of markers. This experimental design led us to the identification of nine QTLs controlling SO<sub>2</sub>. Four of them present in MCH1, STR2 and SSU1 genes were molecularly validated. These alleles also show a pleiotropic effect with link between the production of SO<sub>2</sub> and acetic acid. In the future, these new alleles can be used in cross breeding programs for the improvement of industrial strains.

## II.O.3

## IMPACT OF NON-SACCHAROMYCES IN MALOLACTIC FERMENTATION OF WHITE AND RED WINEMAKING

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**Keywords:** non-Saccharomyces, malolactic fermentation, *Oenococcus oeni*

Nowadays the use of non-Saccharomyces as starters of alcoholic fermentation (AF) has increased because of the modulation of the organoleptic profile of wines. Additionally, these wines can undergo a malolactic fermentation (MLF) driven out by lactic acid bacteria, mainly *Oenococcus oeni*. Since MLF is usually performed after AF, MLF is highly influenced by the metabolism of the yeasts that have conducted the AF.

In the present work, we tested the oenological impact of sequential AF with *Torulaspora delbrueckii* or *Metschnikowia pulcherrima* with *Saccharomyces cerevisiae* on the MLF. Grape musts of Macabeu and Cabernet Sauvignon from 2018 vintage were inoculated with the two non-Saccharomyces. After 48h, the fermenting musts were inoculated with *S. cerevisiae*. Musts inoculated with only *S. cerevisiae* were used as control. After AF, wines were racked and stabilized at 7°C for a week. Two *O. oeni* strains were used to perform MLF of wines corrected in L-malic acid concentration and pH. Also, a spontaneous MLF was followed. General oenological parameters, volatile and phenolic compounds, organic acids and AF and MLF kinetics were studied.

Generally, wines were chemically similar, being the ones fermented with *T. delbrueckii* more different. In all AF the non-Saccharomyces imposition was >90% at 48 h but at the end of AF stage *S. cerevisiae* is the sole dominant species. Moreover, the MLF finished earlier when a non-Saccharomyces was previously been inoculated. In this way, MLF of red wines was already completed spontaneously when AF finished. All MLF finished in less than 8 days with the exception of the spontaneous one in *S. cerevisiae* wine (17 days). Overall, the inoculated MLF were quicker than the spontaneous MLF, apart from an inoculated *O. oeni* strain in *M. pulcherrima* wine. Citric acid was completely consumed after MLF except in the spontaneous MLF of *S. cerevisiae* wine. According to the volatile analyses, the fermentation with *T. delbrueckii* lead a reduction of medium-chain fatty acid concentration. The sensorial analyses showed that the lactic character was highly noticed by the testers in the spontaneous MLF, highlighting the one of *M. pulcherrima* sequential AF.

To sum up, MLF was highly influenced by both the AF strategy (presence of non-Saccharomyces) and the strain of *O. oeni*. Wines obtained with *T. delbrueckii* seem to be more MLF friendly, allowing quick MLF and developing wines more different from *S. cerevisiae*, being the best rated by the testers.

## II.O.4

## MATHEMATICAL MODELING OF FERMENTATION KINETICS: A TOOL TO BETTER UNDERSTAND INTERACTIONS BETWEEN *TORULASPORA DELBRUECKII* AND *SACCHAROMYCES CEREVISIAE* IN MIXED CULTURES

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**Keywords:** modeling, interaction, *Saccharomyces*, *Torulaspora*

Nowadays the use of *Torulaspora delbrueckii* is more and more common in winemaking. However, its behavior in presence of *Saccharomyces cerevisiae* is not always predictable. Indeed, the interactions existing between the two yeasts are still not well characterized and can lead to a bad control during their implementation in mixed cultures. The objective of the work presented here was to use the mathematical modeling as a tool to better understand microbial interactions in this context.

Mixed cultures of a couple of oenological yeasts composed of *T. delbrueckii* and *S. cerevisiae* were carried out on a synthetic grape must in anaerobiosis. The impact of various parameters was evaluated: assimilable nitrogen concentration, direct and indirect contact (thanks to a membrane bioreactor), increase of lipids concentration (Tween 80 and ergosterol).

The analysis of experimental data acquired during the pure cultures of each yeast enable to establish a mathematical model to describe the fermentation kinetics for pure cultures. Then this model was used to predict the kinetics of mixed cultures without any interaction except competition for substrates (sugar and nitrogen). The comparison between predicted and experimental kinetics showed that in mixed culture several kind of interactions must be taken into account: competition for space, cell to cell contact, reciprocal stimulation. Moreover, at low lipids initial concentration, *S. cerevisiae* dominated *T. delbrueckii* by producing a toxic metabolite. An increase in the initial lipids concentration completely reversed this domination.

## II.0.5

### NITROGEN METABOLISM IN *KLUYVEROMYCES MARXIANUS* AND *SACCHAROMYCES CEREVISIAE*: TOWARDS A BETTER UNDERSTANDING OF FERMENTATION AROMA PRODUCTION

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**Keywords:** Nitrogen metabolism, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, Fermentative aroma compounds

During wine alcoholic fermentation, yeasts produce volatile aroma compounds from sugar and nitrogen metabolism. Some of the metabolic pathways leading to these compounds have been known for more than a century. Yet, the differences in compound yield and nature between species remain poorly understood. Using a two-pronged approach of isotopic filiation and transcriptome analysis, this study endeavoured to shed new light on the utilisation of nitrogen sources by two wine-related yeast species, *Saccharomyces cerevisiae* Lalvin EC1118® (Lallemand) and *Kluyveromyces marxianus* IWBT Y885.

The data showed that, although the order and intensity of uptake of nitrogen sources was broadly similar, those of ammonium and arginine differed. Furthermore, the utilisation of assimilated amino acids also differed significantly. While *S. cerevisiae* redistributed the nitrogen in these amino acids evenly for the production of other amino acids, *K. marxianus* clearly favoured specific amino acids. As for amino acids used as substrates for the production of aroma compounds, the fate of leucine and valine did not differ significantly between the two species. However, phenylalanine metabolism differed, and a larger proportion of phenylalanine was channelled through the Ehrlich pathway in *K. marxianus*, resulting in increased production of phenylethanol. Transcriptome data suggest that this shift can be explained by the higher expression of aromatic amino transferases in *K. marxianus*. Taken together, the data show that metabolic pathways are broadly conserved, but that individual nitrogen sources are not always assimilated and metabolised in identical ways. The study also provides new insights on the modulation of fermentative aroma profiles by yeast species of commercial interest.

## II.O.6

USE OF ANTISENSE RNA TECHNOLOGY TO MODULATE GENE EXPRESSION IN *OENOCOCCUS OENI***Cosette GRANDVALET**

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**Keywords:** *Oenococcus oeni*, lactic acid bacteria, antisense RNA , stress response

*Oenococcus oeni* is a wine-associated lactic acid bacterium performs the malolactic fermentation, which improves the taste and aromatic complexity of many wine. Although, wine exhibits harsh and challenging conditions (low pH, low temperature, nutrient-poor and presence of ethanol), *O. oeni* possesses a remarkable adaptability to those physiochemical conditions.

Mechanisms for responding to environmental changes are universally present in living beings and are essential for coping with the stress and for adapting to the new conditions. *O. oeni* tolerance to low pH and ethanol make its an interesting bacteria model for investigating stress response mechanism in lactic acid bacteria. However, lack of appropriate techniques to manipulate *O. oeni* genome has long delay molecular study of this fastidious bacterium. To get around the lack of genetic tool for gene replacement, we focused our work on gene inactivation by using antisense RNA approach to modulate gene expression. With the goal to understanding the function of *O. oeni* hsp genes *in vivo*, we produce antisense RNA targeting genes encoding: a small Hsp (hsp18)1, the master regulator of stress response (ctsR)2 and two caseinolytic protease L members of the HSP100 chaperone family (clpL1, clpL2). Thereby, we highlighted that *in vivo* inhibition of the expression of some of these genes strongly affects the survival of *O. oeni* in stress conditions.

This study presents an elegant approach providing access to an *in vivo* study of gene function in *O. oeni*.

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# Enological Practices and Process

### III.O.1

## GRAPE SEED POWDER AS AN ALTERNATIVE TO BENTONITE FOR WINE FINING

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**Keywords:** grape seeds, bentonite, fining, hazing proteins

PR proteins can cause haze in wines, and the risk is to keep the wine unsold. Generally, in winemaking bentonite solves this problem by removing proteins, but it is not a renewable resource, has poor settling, which means difficulty in filtering after use and a considerable loss of wine, it is not a specific adsorbent and may reduce aromas and flavor compounds. This work studied the use of grape seeds powder (GSP) to remove haze-forming proteins from wine and grape juice. GSP was tried both roasted 180°C x 10 minutes and unroasted, while contact time was set at one hour and two hours for comparison. GSP was tried first on four different heat-unstable wines in small-scale experiments. The results showed that GSP removed PR proteins and permitted to achieve heat stability (DNTU<2) but with high doses (25-32 g/L) of addition. A similar reduction of PR proteins was obtained in all the wines after 1-h contact time with unroasted GSP as well as with roasted GSP, which suggests that roasting did not substantially alter the protein-binding capability of GSP. Contact time (1 or 2 hours) did not change the efficacy of protein removal suggesting that the reaction between grape tannins and proteins occurs within one hour. Treated wines showed changes in the matrix composition, with increased phenolic contents (A280) and improved yellow color (CIELAB b\* parameter). As for the experiments with grape juice, GSP was added in two juices before fermentation to observe the impact on the composition of the finished wines. Roasted GSP was chosen as the fining agent and the contact time was 1 hour. A lower amount of GSP (5 g/L) was observed to be needed to heat-stabilize (DNTU<2) the juices. The corresponding wines showed minor changes in the matrix composition, perhaps because of phenolic-protein interaction and precipitation during the fermentation or degradation via non-enzymatic processes. These results suggested that GSP may be a viable alternative to bentonite. Furthermore, being a by-product of winemaking, GSP utilization would improve the environmental sustainability of winemaking processes.

## III.O.2

## FOAMABILITY OF BENTONITE TREATED WINES: IMPACT OF NEW ACACIA GUM FRACTIONS OBTAINED BY IONIC EXCHANGE CHROMATOGRAPHY (IEC)

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**Keywords:** Acacia senegal gum, sparkling wine, Ionic Exchange Chromatography, foamability

Foam is a key aspect of quality of sparkling wines. Bentonite is usually added to the wine to prevent protein haze, but reducing its foamability [1]. New skills are searching to avoid this undesirable event [2]. *Acacia senegal gum* (*Asen*) is an exudate from *Acacia trees*, which can be used to stabilize red wine color. *Asen* can be fractionated, and the most widely used method is Hydrophobic Interaction Chromatography (HIC) to obtain low (HIC-F1), medium (HIC-F2) and high (HIC-F3) molar mass fractions. The effect of these fractions on the foamability of bentonite-treated wines was studied, showing positive or negative effects depending on the fraction and the wine [3].

*Asen* can also be fractionated by Ion Exchange Chromatography (IEC) giving a high (IEC-F1) and low (IEC-F2) molar mass fractions [4]. A synthetic wine (SYWI) was prepared (12% v/v ethanol, 3 g·L<sup>-1</sup> of tartaric acid). 8 base wines from Spain (3) and France (5) were made by the traditional white winemaking method. They were treated with bentonite (20 g·hL<sup>-1</sup>), stirred gently for a few hours, kept in cold storage (10 days, 4° C), racked and filtered (1 µm). IEC-fractions were added to SYWI (60 g·hL<sup>-1</sup>) and to wines (30 and 10 g·hL<sup>-1</sup>). The foaming parameters were compared by shake test and by a classical gas-sparging method (Mosalux), being the qualitative aspect of foam also observed.

In SYWI, IEC-F1 improves the foamability during the total shake test. Both fractions enhance its Maximum Foam Height (HM) and the Foam Stability Height at 5 minutes (HS) measured by Mosalux. IEC-F1 provides less compact foam with larger bubble. In Spanish wines, IEC-F1 increases the foamability during the total shake test. IEC-F1 also improves it in French wines, but weaker and differently depending on the wine. The foamability is punctually enhanced by IEC-F2 in some wines, but it is greatly decreased in 1 French wine. The dose reduction decreases the improving impact of IEC-F1 on the foamability of the French selected wine but not in the Spanish selected wine. IEC-F1 increases HM and HS in both selected wines, whereas IEC-F2 improves HS only in the Spanish selected wine.

Concluding, the addition of IEC-F1 increases foamability for all the studied wines, but very differently depending on the wine. IEC-F2 addition shows positive, neutral or even negative effects depending on the wine. Dose of IEC-F1 may also play a key role depending on the wine.

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### III.O.3

## OPTIMIZING THE USE OF BENTONITE FOR BETTER CONTROL OF HAZE FORMATION IN WHITE AND ROSÉ WINES

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**Keywords:** wine, protein, haze, bentonite

In winemaking, the appearance of turbidity in white and wine is a serious visual defect, which lowers significantly its commercial value. A major cause of the formation of turbidity in wine is attributed to the presence of temperature-sensitive proteins. The proteins undergo slow conformational changes, leading to aggregation and flocculation phenomena. The process can be accelerated by exposure of wine to high temperatures during transportation or storage. In recent years heat-unstable proteins in white wine were identified as grape class IV chitinases,  $\beta$ -glucanases and a fraction of thaumatin-like proteins. Although proteins play a central role in the formation of turbidity, other components present in wine such as polyphenols, sulfate anion, polysaccharides as well as ionic strength and pH value play an important role in these phenomenon.

The lack of reliable tests assessing the risk of protein clouding during bottle storage is a recurring problem of winemakers. Currently used test assessing haze potential involves heating which often causes overdosing of fining agent. Despite the large progress in the white wine research and substantial development of the analytical methods applied the phenomenon of white wine haze formation remains unrevealed. The traditional treatment used to stabilize wine includes the addition of bentonite, which is certainly effective but due to its non-specific binding results in a considerable decrease in aroma compounds and therefore the quality of the wine. Thus, a strong need to establish a more selective and economically justified method of wine stabilization, which will preserve the aroma compounds in white and rosé wine, is undeniable.

This study aimed at the development of more reliable haze potential tests and more specific treatments for wine. To achieve this objective the knowledge the protein binding properties of different types of commercial bentonite have been analyzed, including the following: elemental analysis, surface charge density, swell index, external and internal specific surface area. The effect of quality of water used for hydration and wine pH on the swelling properties of bentonite have been also investigated. The proteins and polyphenols bound by different types of bentonite as well as the quality of the obtained wine (aroma compounds) have been identified using the above-mentioned methods and compared during three harvest periods. Finally, we have established the possibility of using bentonite alternatively on must with the development of a specific test to establish the dose of treatment.

## III.O.4

## SIMPLIFYING THE MEASUREMENT OF DIFFERENT FORMS OF CU IN WINES AND STRATEGIES FOR EFFICIENT REMOVAL

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**Keywords:** Copper measurement , Sulfide-bound Cu, Filtration , PVP/PVI

Copper (Cu) is known to substantially impact wine stability through oxidative, reductive or colloidal phenomena. Recent work has shown that Cu exists predominantly in a sulfide-bound form, which may act as a potential source of sulfidic off-odours in wine and hence contribute to reductive flavours. The quantification of different forms of copper in wine may allow winemakers to target more effective strategies for the removal of Cu and also to better understand the likelihood of reductive characters emerging in wines during aging.

A simple colorimetric method, utilising bicinchonic acid (BCA), was validated for the determination of the different forms of Cu in white wines, as well as the total Cu concentration in red wine. The determination of total Cu in white wines utilises an addition of excess silver(I) in order to effectively release copper from sulfide and allow quantitative complexation by BCA. The non-sulfide bound form of Cu in the white wine was determined by BCA analysis of the white wine without silver addition. In the case of red wines, a simple digestion procedure eliminated colour prior to subsequent analysis as per the white wines. The total Cu measured by the colorimetric method had an accuracy equivalent to ICPOES and a linear range of 0.04 to 1.0 mg/L. The different forms of Cu measured in white wines agreed with the results obtained by a more laborious electrochemical method.

The removal of different forms of Cu from white and red wine was subsequently studied using membrane filters of various media and pore size, depth filters and PVI/PVP. Only PVI/PVP could efficiently remove both forms of Cu, whilst the filtration techniques displayed activity for removing the sulfide bound form of Cu. Of the membrane filters, nylon and polytetrafluoroethylene media could adsorb sulfide-bound Cu, with little dependence on pore size, but their capacity for removal decreased rapidly with wine filtration volume. Similar results were observed with cellulose-based depth filters, but much greater removal efficiency was observed for cellulose depth filters impregnated with diatomaceous earth. This type of filter had active removal of sulfide-bound Cu from larger volumes of wine. The results allow rapid determination of the Cu forms in wine along with the assessment of the best strategies for their removal.

*Abbreviations: PVI/PVP, polyvinylimidazole/polyvinylpyrrolidone.*

### III.O.5

## BIO-PROTECTION BY ONE STRAIN OF *M. PULCHERRIMA*: MICROBIOLOGICAL AND CHEMICAL IMPACTS IN RED WINES

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**Keywords:** Wine bio-protection, *Metschnikowia pulcherrima*, Metabolomic, Volatile and phenolic compounds

In oenology, bio-protection consists in adding bacteria, yeasts or a mixture of microorganisms on grape must before fermentation in order to reduce the use of chemical compounds such as sulphites.

More particularly, non-*Saccharomyces* yeasts are used as a total or partial alternative to sulphites.

However, scientific data capable of proving the effectiveness of adding these yeasts on grape must remain scarce. A single study in white winemaking showed that early addition of a non-*Saccharomyces T. delbrueckii* strain could be a microbiological and chemical alternative to sulphites (Simonin *et al.*, 2018).

However, there is a lack of scientific data concerning red winemaking where the process allows to leave the yeasts added during the whole winemaking. This study reports for the first time the analysis of microbiological and chemical effects of one *Metschnikowia pulcherrima* strain, inoculated at the beginning of the red winemaking process in three wineries as an alternative to sulphiting. The implantation of the *M. pulcherrima* was successful in all the wineries and effectively limited the development of spoilage microorganisms in the same way as the addition of sulphites. The addition of non-*Saccharomyces* strain could protect must and wine from oxidation as demonstrated by the proanthocyanidin and anthocyanin analysis.

Adding *M. pulcherrima* had no effect on wine volatile compounds and sensorial analysis. However, the untargeted analysis by FTICR-MS highlighted a bio-protection signature and an activation of certain metabolic pathways.

## III.O.6

## GRAPE SOLIDS: NEW ADVANCES ON THE UNDERSTANDING OF THEIR ROLE IN ENOLOGICAL ALCOHOLIC FERMENTATION

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**Keywords:** grape solids, sterols, yeast, alcoholic fermentation

Residual grape solids (suspended particles) in white and rosé musts vary depending on the clarification process. These suspended solids contain lipids (more especially phytosterols) that are essential for yeast metabolism and viability during fermentation in anaerobic conditions. We present here recent advances on the understanding of the role of the solid particles during alcoholic fermentation in liquid phase.

A microscopic approach (epifluorescence with fluorescent probes, confocal and electronic microscopy) allowed to better characterize grape solids physical-chemical structure and lipidic fraction. Then, the lipidic composition of grape solids from white, rosé and red winemaking processes was analyzed.  $\beta$ -sitosterol was the main sterol, but there was considerable variability of overall sterol content of solids depending on their origin. Must turbidity was not representative of sterol content. The use of mutant strains allowed us to provide a model of sterol assimilation in *Saccharomyces cerevisiae* cell. Finally, experimental fermentations were performed in synthetic musts supplemented with the studied grape solids. Sterol content was determinant for the fermentation kinetics when lipids were the limiting yeast nutritional factor.

This impact can be explained by the fact that sterols i) favor yeast nitrogen assimilation and consequently cell growth and maximum fermentation rate, ii) improve cell viability at the end of fermentation reducing risks of sluggish fermentation. Thus, taking into account the sterol content of the must should allow wine-makers to improve control of white and rosé fermentations.

### III.O.7

## CHARACTERIZATION AND APPLICATION OF SILICON CARBIDE (SiC) MEMBRANES TO OENOLOGY

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**Keywords:** Silicon Carbide, Ceramic membranes, Characterization, Filtration

After fermentations, the crude wine is a turbid medium not accepted by the consumer therefore, it needs to be filtered. Wine is increasingly filtered on microfiltration membranes but the low porosity of membranes currently used limits the efficiency of industrial installations. In fact, an increase in flow rates is expected in order to reduce the number of cleaning cycles and the size of the installations.

SiC membranes have very interesting physico-chemical characteristics: low density, high porosity, high hydrophobicity, and good resistance to extreme pH and can become a solution to the problems encountered in the oenology sector. In order to apply these membranes, it's essential to know their microstructure to understand their physico-chemical and hydrodynamic properties. To provide relevant information, different analytical techniques such as 2D, 3D imaging, porosity by mercury intrusion and measurement of contact angle were used. Poral phase analysis of membranes obtained has given concurrent results for all analytical techniques used and with the data provided by the membrane manufacturer. Compared to other ceramic membranes used in oenology, SiC membranes are anisotropic, have a higher porosity (> 40%) and have a lesser tortuosity (1.20) giving them higher permeate flows.

They also have a high hydrophobicity (water = 85.5°) explaining their better resistance to organic adsorption. Due the fact that wine is a complex and fouling colloidal matrix, filtration tests have been carried out on wine in order to identify the best filtration operating conditions. For a turbulent flow regime and a trans-membrane pressure around 2 bars, a high permeate flux was obtained (450 l.h<sup>-1</sup>.m<sup>-2</sup>.bar<sup>-1</sup>) and this flux is permeate flux dependant.

Finally, SiC membranes regeneration was studied: due to an organic fouling found after the filtration sessions, a sodium hydroxide clean-in-place combined with surfactants and hydrogen peroxide at high temperature allowed to recover the total permeability of the membranes.





# Analysis and composition of grapes, wines, wine spirits

#### IV.O.1

### A TOOL FOR CATCHING MICE IN WINE: DEVELOPMENT AND APPLICATION OF A METHOD FOR THE DETECTION OF MOUSY OFF-FLAVOUR COMPOUNDS IN WINE

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**Keywords:** Off-flavour, Mousiness, HPLC-MS, Wine fault

Over the past two years, the AWRI has received 69 wine samples suspected of being affected by mousy off-flavour. The character has been mostly observed in white wines. Possible reasons for this could be the increased use of white winemaking techniques such as high grape solids ferments and extended lees ageing to add textural components to white wine, and higher pH, lower sulfur dioxide and minimal clarification or filtration practices.

Mousy character is an off-flavour in wine that has been described as similar to the smell of caged mice. Although generally infrequent, its detrimental effect on wine quality can cause economic loss to wine producers and, in severe cases, can render wine unpalatable. Mousy off-flavour is a unique wine fault which, due to its chemical nature in wine pH, is rarely perceived by aroma but instead is detected retronasally after affected wine is swallowed or expectorated. There is a wide variation in the ability or sensitivity of individuals to perceive this character, with some tasters unable to perceive it at all. This creates problems for wine producers if they do not have the ability to detect the character during production and therefore do not take remedial action.

The compounds responsible for this off-flavour in wine reportedly include 2-acetyltetrahydropyridine (ACTPY), 2-acetylpyrroline (ACPY), 2-acetylpyridine (AP) and 2-ethyltetrahydropyridine (ETPY). However, the contribution and importance of these individual compounds to mousiness in spoiled wines has not been demonstrated. The unavailability of a practical and reliable method for the detection and quantification of mousy-related compounds in wine has impeded objective measurement of mousy-affected wines and further research in preventing or reducing the occurrence of this fault in wine.

The authors have recently developed a HPLC-MS method for the quantitation of ACTPY, ACPY and AP in wine. The method is simple and rapid and requires only filtration and basification for sample preparation. The analytical run time is approximately 17 minutes for one sample. Precision and accuracy tests confirm that the method is highly reliable and robust. The AWRI has implemented the developed method as a tool for the investigation of wines suspected of being affected by mousiness. A description of the method development and its application to off-flavour investigations will be presented and discussed.

## IV.O.2

## TRIALS WITH MACHINE HARVESTED SAUVIGNON BLANC: THE IMPORTANCE OF GRAPE TRANSPORT TIME AND TEMPERATURE

**Katie PARISH-VIRTUE**

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**Keywords:** Transport time, Temperature, Machine harvesting, Thiols, Sauvignon blanc

It is well known that free varietal thiols, in particular 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA), are important constituents to the aroma of New Zealand Sauvignon blanc wines. This along with the popular practice of machine harvesting in New Zealand were the motivation for the following two pilot studies.

Firstly, it was examined if the presence of 3MH and 3MHA could be influenced by a change in transportation time of machine harvested grapes. This came about as it was noticed that some Marlborough wineries process grapes incoming from multiple growing regions. Here, the thiol precursor contents, Glut-3MH and Cys-3MH, of 21 lab scale wines were examined after experiencing different simulated transportation times (0, 1.5, 3 and 4.5 h). Results suggested that significant ( $p < 0.05$ ) increases in the amount of Cys-3MH and Glut-3MH for some of the treatments associated to longer transportation times was possible. However, after fermentation while some of the experimental wines did not display any significant difference between the transportation times trialled, others displayed an opposite (downward) trend for the presence of 3MH and 3MHA across the increasing time points.

Secondly, as machine harvesting can occur throughout the day and night, of which atmospheric changes in temperature are anticipated, it was hypothesised that the skin contact taking place due to the nature of the machine harvesting can occur at different temperatures. For this study a holding period of 2h was chosen to represent the transport time of harvested grapes to a processing winery while the grape holding temperatures investigated were 6, 15 and 24°C. Cys-3MH and Glut-3MH were quantified both before and after the different temperature treatments of the machine harvested grapes. ANOVA and Tukey HSD did not reveal any significant ( $p > 0.05$ ) differences in thiol precursor levels before the 2h holding period. However, after this time a significant difference ( $p < 0.05$ ) between the 6 and 15°C for both Cys-3MH and Glut-3MH was established. Following fermentation, the levels of 3MH and 3MHA were also quantified and revealed similar levels of these thiols between all of the experimental wines with no significant differences ( $p > 0.05$ ) detected between the holding temperatures investigated.

#### IV.O.3

### HPLC-MS ANALYSIS OF CAROTENOIDS AS POTENTIAL PRECURSORS FOR 1,1,6-TRIMETHYL-1,2-DIHYDRONAPHTHALENE (TDN) IN RIESLING GRAPES

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**Keywords:** Carotenoids, TDN, Riesling, HPLC-MS

In recent years, an undesirable premature "aged" character has been noticed in a growing number of young Riesling wines, associated with extreme weather conditions leading to increased radiation intensity and/or sun exposure of grapes. One of the compounds responsible for rapid aging is 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), a grape derived C<sub>13</sub>-norisoprenoid formed as biodegradation product of carotenoids that participate in light harvesting and are essential for photoprotection against excess light in the blue and green wavelength region (350–550 nm).

Our interest in carotenoids as aroma precursors led us to examine the effect of qualitative light manipulation in the vineyard by coloured shade cloth (green, red and black) on carotenoid profile and accumulation in grapes during the ripening season. Through wavelength modulation of the radiation reaching the vines and therefore regulate the key absorbance maxima of the carotenoids, it was possible to reduce TDN concentrations in finished wines.

This presentation describes HPLC-MS analysis of carotenoids in grapes and will focus on selected carotenoids potentially associated with the formation of TDN.

## IV.O.4

## THE LIMONENE-DERIVED MINT AROMA COMPOUNDS IN RED WINES. RECENT ADVANCES ON ANALYTICAL, CHEMICAL ASPECTS AND SENSORY ASPECTS

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**Keywords:** mint aromas, red wine, aging, terroir

In recent years, the ageing bouquet of red Bordeaux wines has been partially unveiled by a chemical and sensory point of view<sup>1-3</sup>. Minty and fresh notes were found to play a key role in the definition of this complex concept, moreover the freshness dimension in fine aged red wines plays an important role in typicity judgement by wine professionals. Piperitone, a monoterpene ketone, was identified as a contributor to the positive mint aroma of aged red Bordeaux wines<sup>4,5</sup>. Further chemical and sensory investigations led to identification of a pool of mint aroma compounds (i.e. p-menthane lactones, carvone and menthol) potentially responsible for these positive olfactory notes.

The analyses of Merlot and Cabernet Sauvignon wines from various terroirs of the Bordeaux area suggested that there was a varietal influence on the mint aroma compound profiles<sup>5</sup>. Recently, a study in which we defined the terpenic profile of the two Italian grape varieties Corvina and Corvinone, confirmed that the concentration of the mint compounds is variety dependent, despite the terroir of origin of grapes.

These results revealed that Corvina wines were significantly richer in the pool of minty terpenes, in all the considered terroirs. Our recent results also revealed that these compounds already exist in the young wines, but at lower concentrations than in aged ones, thus suggesting that the mint compounds in wine reveal themselves during ageing. The mechanisms of this revelation are still unclear and are today studied.

The results of the last years have opened the way to many questions that are still not answered and require further studies, in particular the role of the soil, viticultural practices, climate, rootstocks and varieties must be investigated. The determination of these compounds in wine is quite complex, as they are present at ng/L levels; however, they are sensory active also at trace levels, due to their low perception thresholds and synergistic sensory effect<sup>4</sup>.

The coupling of HS-SPME Arrow extraction and GC-MS-MS analysis has permitted to develop and validate an automated method of quantification. The development of this simple, sensitive and accurate analytical methods will allow to analyse large sets of wine, thus deepening the knowledge on the origin and expression of the minty and fresh aromas in wine, one of the most important piece of the puzzle of the ageing bouquet.

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#### IV.O.5

### EVOLUTION OF THE CROWN PROCYANIDINS DURING WINE MAKING AND AGING IN BOTTLE

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**Keywords:** Crown procyanidins, Condensed tannins, Wine making, Wine aging

Condensed tannins are widely distributed in plant-derived foods and beverages like grape, red wine, nuts, tea, apples and chocolate in which they contribute to multiple sensorial properties such as flavor, color, and taste (astringency and bitterness). During the wine making process, condensed tannins are extracted from the skins and seeds of the grape and their concentration in red wine are influenced by the grape varieties as well as technical process used. Recently, a condensed tannin sub-family with an unusual skeleton has been reported, and named crown procyanidin. These compounds have a specific structural feature of being cyclic and being composed only of B type inter-flavonoid linkages. All the sub-units of the NMR characterized tetramer are (-)-epicatechin and it presents within its structure a relatively big cavity and composed of four aromatic rings and several phenol functions. The first identification of the tetramer and some pentamers has been report in the red wine. However, recent study showed that these molecules are specifically located in grape skins and there concentration in red wine depends of the grape varieties.

The goal of this study was to determine the evolution kinetics of crown procyanidins (tetramer and pentamers) during the wine making process as well as during the aging of red wine in bottles. The organoleptic impact of these new tannins sub-family has been also investigated.

Firstly, the extraction evolution kinetics of crown procyanidins was determined during the wine making of Carbernet Sauvignon grape harvested in Pauillac, Bordeaux. It appears that the crown procyanidins are extracted at the beginning of the maceration and are highly water soluble in contrast of non-cyclic condensed tannins which need alcohol to be extracted. Indeed 70% of crown procyanidins are extracted during the first 24 hours. Secondly, red wines aged between 2 to 20 years have been obtained from the same wine-ry and sensorial analyses as well as chemical analysis have been performed on an UPLC-UV-QTOF. During red wine aging in bottle, the crown procyanidins concentrations remain stable, whereas the noncyclic condensed tannins decreased with time. In order to understand why the concentration of crown procyanidins remain stable, some oxidisability test has been performed to compare the crown procyanidins and the noncyclic condensed tannins and the crown procyanidins appeared to be more resistant to oxidation than noncyclic condensed tannins.

## IV.O.6

## THE FUNDAMENTAL ROLE OF PH IN THE ANTHOCYANINS CHEMICAL BEHAVIOR AND IN THEIR EXTRACTABILITY DURING WINEMAKING

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**Keywords:** Anthocyanin , pH, pigmentation, anthocyanin solubility

The chemical behavior of anthocyanins is considerably affected even by slight pH variations with important implications for the winemaking as well as for the wine conservation. Considering that this is a central issue to the enological sector, we decided to better investigate the influence of pH on the anthocyanin chemistry. Initially, by chromatographic and advanced NMR techniques the chemical behavior of malvidin-3-O-glucoside was studied in wine-like solutions with pH values ranging from 3 to 4. First, the already composite aqueous equilibrium of malvidin-3-O-glucoside turned out to be even more complex than so far thought, as a new hydration product of the anthocyanin was detected and characterized in solution. More importantly on account of its technological implications, the anthocyanin solubility appeared to decrease remarkably as the pH value of the wine-like solutions increased. A dramatic drop in terms of anthocyanin solubility was observed at pH 3.32, where the measured molecule concentration was reduced to almost 25% the expected one. Also, at such pH level the anthocyanin self-association appeared significantly affected. In more detail, the flavylum ion self-association predominant at lower pH levels was altered and found to co-occur with a preferential co-pigmentation involving flavylum ion species and the trans-chalcone form of malvidin-3-O-glucoside. At higher pH values, this latter association was the only one detected in solution. In the light of these results, we set up an experimental protocol with the purpose of analyzing the pH influence on the anthocyanin chemistry and extractability in real wines produced by varying their pH levels during the maceration-fermentation phases. Preliminary chemical analysis of such wines provided data consistent with those obtained in wine-like solutions. Indeed, the extraction of malvidin-3-O-glucoside and that of anthocyanins in general was more contained as the pH value increased. Additionally, other molecules of enological interest, including catechins, cinnamates, syringic acid and terpenoids, turned out to be affected by the wine pH. These analytical data highlight the fundamental role of pH during the winemaking and the importance of regulating its level to obtain wines with the desired polyphenolic structure.

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#### IV.0.7

### WINE FINING WITH YEAST PROTEIN EXTRACT: EFFECT ON POLYPHENOL COMPOSITION AND THE RELATED SENSORIAL ATTRIBUTES

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**Keywords:** fining, yeast protein extract, organoleptic properties, salivary proteins

Polyphenols, namely anthocyanins and flavanols, are key compounds for wine color definition and taste perception (astringency and bitterness). During winemaking, several processes could influence the polyphenol composition and, therefore, the organoleptic parameters of wine.

It is widely known that fining can remove soluble substances, including polymerized tannins and coloring matter in red wines affecting the organoleptic properties of wine. The use of yeast protein extracts (YPE) as fining agents arises from the allergic properties observed in classic protein-based fining agents. From the oenological point of view, it was already verified that this new fining agents promote the clarity and stabilization of wine, however is still a gap in their influence in polyphenol composition and the related organoleptic properties.

The aim of this work is to understand the molecular mechanisms as how YPE-wine polyphenols interactions could modulate the color as well as the taste sensations (astringency and bitterness) after wine fining with YPE (developed by Proenol, Biotechnology Industry). Briefly, polyphenols related with astringency and bitterness were analyzed by LC-MS. The influence of YPE on wine color was also assayed by CieLab system. Furthermore, the effect of YPE-wine on the interaction with salivary proteins was also analyzed by SDS-PAGE after wine ingestion during a sensory evaluation. Finally, the results from experimental data were compared with the results obtained by sensorial panel.

Overall, it was concluded that wines clarified with YPE revealed a significant decrease in the majority of identified compounds related to bitterness and astringency. The study of wine color revealed that YPE had the ability to reduce yellow color of white wines and did not remove red color of red and rosé wines, which is an important aspect in consumption market. Besides the decreasing of several polyphenols related with taste perception, some relevant differences were observed in the salivary protein profile by SDS-PAGE. The results observed herein highlighted the relationship between (1) the taste perception, (2) the interaction between salivary proteins and wine polyphenols during the sensorial evaluation and (3) the effect of YPE fining in wine polyphenols.

In summary, YPE reveals to be a good alternative to protein animal origin fining agents due to the ability to promote wine sensorial properties.

## IV.O.8

**BEYOND COLORS OF ROSÉ WINES: IMPACT OF ORIGIN AND WINEMAKING TECHNOLOGY ON THEIR COLOR, POLYPHENOL AND THIOL COMPOSITIONS****Cédric SAUCIER**

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**Keywords:** Rosé wine, polyphenomics, thiols, PVPP fining

Rosé wine consumption is rapidly increasing with its market share in France that has grown from 11 % to 32 % in less than 20 years. A recent trend is also to produce rosé wines with lighter colors. Varieties, terroir and technology certainly have an influence on rosé wine colors. We used different analytical techniques (colorimetry, UPLC-MS) and data management strategies (molecular modelling and multivariate discrimination analysis) to investigate the relationship between natural and human factors on the final composition of rosés wine. We showed that some polyphenols can be key markers of the origin for 60 commercial wines from the Bordeaux, Languedoc and Provence regions. We also demonstrated that PVPP treatment reduces the color of rosé wines by specifically adsorbing some classes of polyphenols and pigments like coumaroylated anthocyanins. This specific adsorption phenoma was explained by molecular modelling calculations of interactions between anthocyanins and PVPP. Finally we showed for the first time that the thiol aromatic indexes of rosé wines can be increased by PVPP treatment up to 200 % compared to the control.

#### IV.O.9

### CHANGES IN RED WINE COMPOSITION DURING BOTTLE AGING: IMPACTS OF VITICULTURAL CONDITIONS AND OXYGEN AVAILABILITY

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**Keywords:** bottle ageing, viticultural conditions, oxidative-reductive development

Bottle ageing is considered essential for most premium red wine production. An important aim of bottle ageing of wine is to achieve a balance between the oxidative and reductive development. This is typically evaluated by the accumulation of aldehyde compounds (causing oxidative off-flavour) and sulfur-containing compounds (causing reductive off-flavour) in the wine [1]. Although studies have been performed using variable amounts of oxygen available to the wine during bottle ageing, the impacts of viticultural practices on ageing processes have been studied less.

This research investigated the impacts of viticultural conditions (2 grape varieties, 2 vineyard locations and 2 fruit maturities) and variable bottle ageing conditions (3 oxygen availability regimes and 4 bottle ages) on red wine composition, with a particular focus on the production of aldehyde and sulfur-containing compounds in wine. Analysis of the total concentrations of the key aroma compounds was performed by LC-QQQ-MS (aldehyde compounds in their hydroxyalkylsulfonate forms), HS-GC-SCD (sulfur-containing compounds) and HS-SPME-GC-MS (esters, C6 compounds and terpenes). After 24-month of ageing, all of the measured sulfur-containing compounds showed increased concentrations in all wines.

For the wines made from the same grape (i.e. same variety, vineyard and harvest date), the high oxygen availability treatments contained lower concentrations of sulfur-containing compounds compared to the low oxygen treatments. Generally, methional, 2-methylpropanal, 3-methylbutanal, 5-methylfurfural and furfural exhibited increased concentrations with time, while phenylacetaldehyde, benzaldehyde and hexanal showed decreased concentrations.

Chemometrics analysis showed that based on the compositional analysis, samples could be separated primarily based on wine ageing and the viticultural conditions adopted. Regardless of the oxygen regimes or the ageing time during the bottle ageing, the samples could be always separated according to the viticultural conditions of the grape. These results indicate the importance of the viticultural conditions of the grape on the ageing of wine, despite the extent of ageing or oxygen exposure.

Reference:

[1] Ugliano, M. Oxygen contribution to wine aroma evolution during bottle aging. 2013. *Journal of Agricultural and Food Chemistry*. 61(26): 6125-6136.

## IV.O.10

## KEY ODORANTS RESPONSIBLE FOR THE SENSORY SPACES DEFINING THE DIFFERENT AROMA POTENTIALS OF GRENACHE AND TEMPRANILLO GRAPES

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**Keywords:** Phenolic and aromatic fractions (PAFs), accelerated hydrolysis, sensory analysis, GC-O

There are yet many gaps in our knowledge about the aroma potential of winemaking grapes and its measurement. Trying to bring some light into this question, a new general strategy based on the accelerated hydrolysis of reconstituted phenolic and aromatic fractions (PAFs) extracted from grapes has been developed. In this paper, we present results obtained by applying such PAFs strategy to the study of 33 different lots of grapes from grenache and tempranillo from different areas of Spain and different qualities.

Grapes were first crushed and macerated in the presence of ethanol to avoid fermentation. The ethanolic must was pressed and filtered, then, an aliquot was centrifuged, dealcoholized and extracted in a C18 cartridge. Phenols and aroma precursors, PAFs, were eluted with ethanol. This ethanolic fraction was then reconstituted with water and tartaric acid to make a reconstituted wine model (r-PAF; 13.3% ethanol, pH 3.5). Aroma was developed by storing the r-PAFs in complete anoxia at 75°C for 24h. The 33 ar-PAFs were subjected to different sensory analyses. First, a sorting task to define sensory categories and to select the most representative samples, which were characterized by flash profiling and by gas chromatography-olfactometry (GC-O).

Samples developed strong aroma nuances over a background of vegetal and dry fruit odors and were classified into six different sensory categories: 1) citrus & floral; 2) dried fruit & floral; 3) wood, toast & red fruit; 4) red fruit, black fruit & dried fruit; 5) vegetable & dried fruit; and vi) vegetable. Vegetal notes were attributed to aroma compounds derived from lipid oxidation (Z-3-hexenal, Z-2-nonenal, E-2-nonenal and 1-octen-3-one), while the dry fruit background was attributed to  $\beta$ -damascenone and to massoia lactone. Citrus notes were associated to the surprising presence of 3-mercaptohexanol, whose origin has been exclusively associated to fermentation. Woody and toasty character were attributed to guaiacol and 4-allyl-2-methoxyphenol while furaneol and an unknown ester-like odorant could be linked to red fruit notes. Samples from grenache were more often classified as floral, citrus and dry fruit, while samples from tempranillo were more often classified as woody, toasty, red fruit and vegetal.

Overall, the procedure provides a new insight into the aroma potential of winemaking grapes, which should be helpful in understanding and managing grape quality.

**Acknowledgment.** Work funded by the Spanish MCIU AGL2017-87373-C3-1R. Y.A. and LAAE acknowledge the Diputación General de Aragón for a predoctoral fellowship, as well as the European Social Fund.

#### IV.O.11

### IMPACT OF GRAPE MATURITY ON ESTERS CONTENT AND SENSORY CHARACTERS IN WINES FERMENTED WITH YEAST STRAINS OF DIFFERENT GENETIC BACKGROUNDS

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**Keywords :** Wine aroma, Esters, Maturity, *Saccharomyces cerevisiae*

Grapes composition is a factor well known to affect wines composition and sensory expression. The goal of this study was to evaluate how grapes composition modifications linked to maturity level could affect wines aromatic expression and esters composition. An experimental design has been developed from grapes of *Vitis vinifera* cv Merlot. On each vine plot, grapes have been harvested at two maturity levels and then have been fermented under standardized condition with two yeast strains : a commercial one and another obtained by deletion of the four main esterases of the previous one. Fermentation performed with the esterases deleted strain led to wines with main ester levels generally lower by a factor 5 to 10 in comparison with the original strain.

Merlot wines from the highest maturity level and fermented with the commercial strain shown lower concentrations for fatty acids ethyl esters and higher alcohol acetates but higher concentrations for some substituted ethyl esters like ethyl leucate. When fermentations were performed with an esterases deleted strain, all esters contents remained the same.

Sensory analysis confirmed these results. For the wines fermented with the commercial strain, when the maturity increased, wine fruity aromatic expression decreased (particularly its global intensity and the fresh, redberry- and fermentative fruits character) whereas when the fermentation was performed by the deleted strain wines fruity characteristics were the same.

Aromatic reconstitution performed, on one hand, to erase the consequences of maturity differences and, on the other hand, to erase the consequences of the strain performing alcoholic fermentation on esters contents showed that esters were not, alone, responsible for the difference of sensory characteristics for wines from very ripe grapes (particularly for the jammy fruit notes) but that their presence was essential for the perception of this difference.

Our results highlight once again the role of esters in the overall wine fruity aromatic expression and underline their indirect importance in the perception of some varietal characteristics through perceptive interaction phenomena.

## IV.O.12

## AROMA CHARACTERIZATION OF AGED COGNAC SPIRITS: CONTRIBUTION OF VOLATILE TERPENOID COMPOUNDS

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**Keywords:** Cognac, aroma, terpene, gas chromatography

Cognac spirit aromas result from the presence of a wide variety of volatile odorous compounds associated with the modalities of distilled spirit elaboration and during aging. Indeed, these odorous compounds play an essential role in the finesse and complexity of the aged Cognac. However, very few studies have been done on this subject especially regarding to aging notes with exotic wood (sandalwood) and balsamic descriptors. The aim of this work is to improve knowledge of these aromatic nuances.

To answer this question, a sensory-guided approach was used after selection by an expert panel of aged Cognac spirits. After solvent extraction, HPLC fractionation was used in order to evidence fractions of interest recalling specific aromatic nuances of aged Cognac. Doing so, it was possible to select some HPLC fractions presenting interesting notes of aged Cognac and recalling particularly exotic wood and balsamic descriptors. Their contribution was demonstrated by reconstitution and omission tests with all HPLC fractions.

Then the GC-O/MS analysis of the selected fractions allowed to detect odorous zones and identify associated compounds. First, various well known compounds representative of terpenoids family were highlighted as geraniol,  $\alpha$ -terpinene, nerol,  $\alpha$ -terpineol, 1,8-cineole (eucalyptol) and particularly piperitone which was not cited in Cognac. These compounds were quantified and their detection thresholds were carried out.

An insight of their evolution during aging shown that geraniol,  $\alpha$ -terpinene,  $\alpha$ -terpineol and 1,8-cineole concentrations increased while spirit were more aged, while nerol tend to decrease. However aging had no impact on piperitone. The impact of wine distillation with lees was also particularly considered and the quantification of these compounds showed an impact of this parameter of the elaboration process.

The organoleptic impact of 1,8-cineole was highlighted in Cognac at concentration over its detection threshold. While continuing the GC-O analysis and its coupling to mass spectrometry, aroma nuances of sandalwood, were particularly noticeable. Thus, other molecules were identified through the sensory-guided method. They were santalol with sandalwood nuances and  $\alpha$ -campholenal with tea and sandalwood nuances, both of which were related to sandalwood, respectively a sesquiterpene and a monoterpene. These new identifications thus open up a large field of investigation concerning the terpenoids family and their sensory impact within the aged Cognac matrix.



# **Chemical and Biochemical reactions, including grape and wines microorganisms impact**

## V.O.1

# WHAT IS THE FATE OF OXYGEN CONSUMED BY RED WINE? MAIN PROCESSES AND REACTION PRODUCTS

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**Keywords:** Oxygen, Acetaldehyde, Polyphenol index, Anthocyanins, flavonols, tannins and flavanol-anthocyanins adducts

Recent works have shown that:

Oxygen consumed by wine is used to oxidize sulfur dioxide and ethanol to form acetaldehyde wine oxygen consumption rate (OCR) was negatively correlated with the initial acetaldehyde level. Experiences carried out at 25°C with red wines have demonstrated that after consuming a large amount of O<sub>2</sub>, some young wines did not form acetaldehyde. However, acetaldehyde level increased in aged wines. Higher acetaldehyde accumulation in aged wines can be explained by Aldehyde Reactive Polyphenols (ARPs) smaller amounts, because of their lower reactive potential due to high O<sub>2</sub> exposure. Models characterized ARPs as anthocyanins, flavonols, tannins and flavanol-anthocyanins adducts. These ARPs should be closely related to wine aging potential by measuring acetaldehyde consumption rate (ACRs) and/or the maxima amounts of acetaldehyde each wine can consume.

The main goal of this work was to find a new polyphenol index which should be linked to wine oxygen consumption kinetics. It could indicate the maximum oxygen level that a wine can consume. As well as, elucidate if acetaldehyde is the reactive species with ARPs, but one of its radical precursors in the Fenton reaction.

Three experiments were prepared in anoxia followed by total acetaldehyde determination by using HPLC: 1) wines spiked with 30 and 300mg/L of acetaldehyde and incubated at 25, 45 and 70°C; 2) synthetic wines spiked with 15 to 120mg/L of acetaldehyde and polyphenol extracts; 3) synthetic matrices filled with malvidin-3-O-glucoside, catechin and a mix of both, which were exposed to: a) 8 mg/L O<sub>2</sub> to form acetaldehyde in situ or b) to anoxia and spiked acetaldehyde (11mg/L).

Several wines consume acetaldehyde at different rates, which are particularly imprecise at low temperatures. This makes impractical the use of ACRs as an index to categorize wine polyphenolic composition by defining a discrete ARP category. ACRs are too complex, showing a high dependence order towards acetaldehyde level and an equilibrium concentration. Such concentrations were found to depend on the previous acetaldehyde uptake by the polyphenolic fraction, but it was too imprecise to take clear conclusions. In any case, measured ACRs are smaller than expected attending to oxygen consumption kinetics and acetaldehyde accumulation rates. No significant differences were found when comparing the acetaldehyde formed in situ or when acetaldehyde was spiked.

Results show that oxygen consumed by wine is used to oxidize SO<sub>2</sub>, ethanol and at least 50% to oxidize ascorbic acid, cysteine, glutathione, H<sub>2</sub>S, thiols, methionine and phenols.

This work has been funded by the Spanish Ministry of Economy and Competitiveness (Spanish FPI Program AGL2014-59840-C2-1-R, AGL2017-59840), by Diputación General de Aragón (T53) and Fondo Social Europeo.

## V.O.2

## IMPACT OF GLUTATHIONE-RICH INACTIVATED YEAST ON WINE CHEMICAL DIVERSITY

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**Keywords:** yeast derivative, glutathione enrichment, metabolomic, peptide diversity

Glutathione-rich inactivated dry yeasts (GSH-IDY) are claimed to accumulate intracellularly and then release glutathione in the must. Glutathione is beneficial to the wine quality, but scientists also highlighted that GSH-IDYs have a greater effect than only increase the pool of this antioxidant in the wine. This work unveils the extent of diversity of compounds potentially released by three different IDYs with increasing GSH contents.

Unsupervised analysis of IDYs released compounds in model wine was performed with the ultra-high-resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). This powerful tool allows to have an instant picture of the released compounds chemical diversity. Bioinformatics strategy (chemometric analysis and network annotation) were then applied to visualize and refine the generated data.

Our results clearly show an impact of the GSH accumulation process not only visible on the glutathione itself, but also on the global diversity of compounds. The ratio of annotated CHONS/CHO ions increased from 0.2 to 2.1 respectively with the accumulation of GSH. The IDY with the highest concentration of GSH released 36 unique CHONS annotated ions compared to the two others IDYs. Since the bioprocess dedicated to accumulate the intracellular glutathione used cysteine rich medium, the possibility to attribute this diversity to notably a larger number of cysteinyl residues in peptides raised. Within the 1699 detected ions by (-)FT-ICR-MS, 193 were annotated as peptide sequences (from 2 to 5 residues). Within this pool of peptides, the IDY specific diversity increased with the level of glutathione from 5 to 45 unique m/z. Besides the global diversity, m/z attributed to cysteine containing peptides were much more abundant in the GSH-rich IDY. Within the 25 peptides containing cysteine, and common to the three IDYs, 64% were more intense in GSH-rich IDY. Thus, the process leading to accumulate glutathione is also involved in other metabolic pathways which contribute to increase CHONS containing compounds and notably peptides.

This work gives new clues on the potential of biotechnology to improve the efficiency of natural yeast derivatives to produce potential active compounds such as cysteine containing peptides. This could lead to substitute partially the chemical additives and thus leading to a better control of wine quality and a better consumer acceptability.

### V.O.3

## ARE DICYSTEINYL POLYSULFANES RESPONSIBLE FOR POST-BOTTLING RELEASE OF HYDROGEN SULFIDE?

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**Keywords:** Polysulfanes, S-sulfonate, Copper, Sulfur dioxide

Hydrogen sulfide (H<sub>2</sub>S) has a significant impact on wine aroma attributes and wine quality when present at concentrations above its aroma threshold of 1.1 to 1.6 µg/L. Therefore, the management of H<sub>2</sub>S concentrations in wines, whether from fermentation or “other” origins, is an important consideration for winemakers. The main techniques used for H<sub>2</sub>S removal are oxidative handling and/or copper fining; however, the effectiveness of these treatments may be temporary, as H<sub>2</sub>S can often reappear post-bottling along with other volatile sulfur compounds (VSCs) when reductive conditions are re-established. Moreover, it is proposed that oxidative treatments applied in the presence of copper may produce compounds such as disulfides and diorganopolysulfanes, which might initially appear stable but are susceptible to reductive cleavage, thereby potentially acting as latent sources of H<sub>2</sub>S.

The aim of this study was to determine whether putative polysulfanes could act as latent sources of H<sub>2</sub>S during post-bottling storage. Experiments conducted in model wine enabled identification of four dicysteinylnyl polysulfanes when H<sub>2</sub>S was oxidised in the presence of cysteine, copper and iron. The stability of the dicysteinylnyl polysulfanes formed in-situ was evaluated and conditions impacting the release of H<sub>2</sub>S from the polysulfanes were also determined, which provided some understanding of the possible mechanisms of release.

The results of this study showed that the stability of the dicysteinylnyl polysulfanes decreased as sulfur chain length increased, which accorded with the relative proportions of polysulfanes initially formed. Notably, H<sub>2</sub>S was released over time, with the greatest decline in polysulfane relative abundance and largest release of H<sub>2</sub>S (up to 212 µg/L) being associated with the addition of commonly used reducing agents, especially sulfur dioxide, to the model wines containing the polysulfanes. Desulfurisation of cysteine could account for only minor quantities of H<sub>2</sub>S. In addition, Cysteine-S-sulfonates were tentatively identified by mass spectrometry after six months of storage, and similarly to the parent polysulfanes, their relative concentrations decreased with increasing number of linking sulfur atoms. These results shed light on the potential pathways for reformation of VSCs in bottled wine and demonstrate that dicysteinylnyl polysulfanes may have the potential to act as latent sources of H<sub>2</sub>S in wine post-bottling, potentially via a sulfitolysis mechanism.

## V.O.4

**NMR APPROACH FOR MONITORING THE PHOTO-DEGRADATION OF RIBOFLAVIN AND METHIONINE****Daniela FRACASSETTI**

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**Keywords:** Light exposure, Nuclear Magnetic Resonance, Oxidation, Tannins

The light exposure of white wine is responsible for several reactions leading to changes on colour, flavours and, consequently, affecting the sensory profile. These reactions can take place when the white wine is bottled in clear glass and their mechanisms are dependent on both light exposure and chemical composition of white wine. Particular attention has been given to the reaction involving riboflavin (RF), a photo-sensitizer compound, and methionine (Met), a sulfur-containing amino acid, that can cause the formation of volatile sulphur compounds (VSCs), namely methanethiol and dimethyl disulfide. These compounds are responsible for a defect known as light-struck taste. Previous studies showed that hydrolysable tannins, in particular those from nut galls, limited both the degradation of Met and the formation of VSCs. The effectiveness of hydrolysable tannins was also proved after light exposure and storage for 24 months.

In order to better understand the role of tannins in the photo-degradative reactions, an NMR approach was carried out. A solution containing RF (0.2 mM) and Met (2 mM) acidified at pH 3.2 was exposed to light by using fluorescence light bulbs. The solution was exposed to light up to two hours sampling it every 15 minutes. The same experimental conditions were applied in presence of gallic acid (2 mM), a constitutive unit of nut gall tannins.

The degradation of RF and Met was monitored and, as expected, their signals decreased as the light exposure increased. Results provided evidence that a new signal appeared at 2.64 ppm. This signal was assigned to the SOCH<sub>3</sub> moiety of methionine sulfoxide through the addition of the standard solution and standard 2D-NMR assignment techniques. The formation kinetic of methionine sulfoxide was measured for increased duration of light exposure and its rate resulted two-folds lower with the addition of gallic acid. This result suggests that the limited degradation of Met in presence of tannins, also observed in previous studies, is due to their action as competitor with Met in reducing RF from its excited form.

The NMR technique was suitable for monitoring the photo-degradative reaction of RF and Met. Further researches have been carried out in order to verify and prove the ability of tannins in quenching both singlet oxygen and RF.

## V.O.5

# STUDY OF YEAST BIOCATALYTIC ACTIVITY ON GRAPE AROMA COMPOUNDS

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**Keywords:** aroma, yeast, terpenes, biocatalysis

Many volatile compounds of different chemical/biochemical origin contribute to wine aroma. Certain key 'varietal' aroma compounds such as methoxy-pyrazines are formed in the grape and appear to be only scarcely influenced by fermentation. Conversely, other grape-derived compounds undergo important transformation during fermentation, so that grape varietal volatile pattern is substantially different than the one observed in the corresponding wine. While this phenomenon is generally regarded from the point of view of the cleavage of glycosidic or amino acidic precursors, recent studies highlighted the existence of other bio-catalytic processes taking place during fermentation, which could be relevant to wine aroma generation. Accordingly, in addition to enzymatic activities such as glycosidase, b-lyase, and/or acetyl-transferase, other enzymes could play a role in the expression of wine varietal aroma typicality, although these have been poorly characterized so far. Certain key volatile such as norisoprenoids (fruity attributes), lactones, (dry fruit attributes), cyclic terpenoids (minty and balsamic attributes), sesquiterpenes and benzenoids (balsamic and spicy attributes) could be associated with such processes. Some of them could also arise from the combination of yeast enzymatic and acidic rearrangements taking place at wine pH.

The aim of this work was to investigate the biotransformation of potentially relevant grape metabolites by *Saccharomyces cerevisiae*. Cyclic, oxydrylated, or ketonic terpenes, sesquiterpenes, aliphatic lactones and aldehydes, hydroxyl acids and benzenoids were all investigated, as well as precursors extracts from different grapes. Biotransformations were screened by placing target compounds under incubation (at 37 °C) with yeast resting cells for 72 hours under variable conditions. After incubation, the products of biotransformation were analyzed by SPME-GC-MS and their aroma evaluated by GC-O.

The results highlighted the occurrence of several complex transformations involving, among others, reduction of allylic carbonyl and carbon-carbon double bond, stereospecific reduction of terpenic ketones, acetylation. These reactions occurring to grape metabolites produced odoriferous molecules considered to participate to the characteristic aroma of some wines. The methodology employed in our study turned out as an effective approach to study the process of aroma generation from neutral grape into wine. As first application, this study has allowed to elucidate some aspects concerning the balsamic notes appearing in wines made with Corvina grapes.

## V.O.6

**MULTI-OMICS METHODS TO UNRAVEL MICROBIAL DIVERSITY IN FERMENTATION OF RIESLING WINES****Kimmo SIREN**

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**Keywords:** Metagenomics, Metabarcoding, Chemical interactions, Machine learning

Wine aroma is shaped by the wine's chemical compositions, in which both grape constituents and microbes play crucial roles. Although wine quality is influenced by the microbial communities, less is known about their population interactions. Previous studies linking the effect of native microbial communities to sensory relevant aroma compounds with their interactive properties have been vastly unsuccessful to date. Partially because studies relied on relatively few isolated strains or chemical compounds, which may be not sufficient to fully understand this complex picture.

Native microbial communities from different Riesling vineyards were studied over multiple experiments during vinification as well as over a two-year to reveal their effects on chemical and sensory composition of spontaneously fermented Riesling wines.

We demonstrate that by combining modern untargeted high-throughput omics technologies and statistical approaches, it is possible to look into samples in situ in the actual natural environment. Our results indicate that both vineyard and winery microbial communities are found to play significant roles in wine. Microbial communities within the fermenting were strongly influenced by vineyard of origin.

These population dynamics are consequently translated into diverse sensory properties through sensory relevant chemical interactions. We found that both sensory and chemical compositions were heavily influenced by the microbial community composition during the vinification as well as the vineyard and the year. Such methodologies allow to find novel microbial and chemical patterns which could be further tested with targeted studies. In addition to deconstructing the microbial community composition in complex natural environment, we leverage on shotgun metagenomic data to undertake the functional analysis of the microbial community during wine fermentation. In the future, multi-omics approaches will be essential for fully discovering the complexity of biological networks, where microbes, host and chemical compounds interact with human sensory perceptions. These developed approaches benefit any industry that works with complex biological interactions.

V.O.7

## OENOLOGICAL TANNINS TO PREVENT *BOTRYTIS CINEREA* DAMAGE: REDUCTION OF LACCASE ACTIVITY

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**Keywords:** Oenological tannins, Botrytis cinerea laccase, Kinetics, SDS-PAGE

### Chemical and Biochemical reactions, including grape and wines microorganisms impact

Oenological tannins are classified as hydrolysable and condensed tannins. Their use in winemaking is only authorized, to facilitate wine fining. Nevertheless, tannins could also be used to prevent laccase effects.

Indeed, our group has recently proved their effects against laccase damage [1]. The goal of this study was to better understand the mechanism of action of oenological tannins on laccase activity induced by Botrytis cinerea. Five oenological tannins were used (gallotannin, ellagitannin, quebracho, grape-skin and grape-seed) and compared with ascorbic acid (AA) and sulfur dioxide (SO<sub>2</sub>). Oenological tannins, AA and SO<sub>2</sub> were added to botrytized must at different doses. After 4 minutes, laccase activity was measured by the syringaldazine method [2] using different concentration of substrate. Enzymatic kinetic constants (Km/Vmax) were determined according to Michaelis-Menten model. Furthermore, *B. cinerea* (strain 213) was grown in a stimulating liquid medium for laccase production [3]. The molecular weight (MW) and the effect of bentonite and tannins upon laccase were studied by SDS-PAGE. The results confirm that all oenological tannins inhibit laccase activity and that the higher the dose the lower the laccase activity. In this way, gallotannin, grape-seed and skins tannins seem to be the most effective tannins. All the tannins were as effective as AA, even though SO<sub>2</sub> was clearly the most effective inhibitor. The laccase produced by *B. cinerea* had a MW of 95 kDa. After bentonite treatment all wine protein bands disappeared and laccase band decreased slightly although its activity remains stable. The interaction between different oenological tannins and laccase was analyzed by measuring the reduction of the intensity of the laccase band. In general, the reduction of band intensity correlates with the reduction of laccase enzymatic activity.

It can be concluded therefore that oenological tannins a good candidate to prevent laccase effects, helping to diminish the SO<sub>2</sub> dose in grapes infected by *B. cinerea*.

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V.O.8

## WHEN ORGANIC CHEMISTRY CONTRIBUTES TO THE UNDERSTANDING OF METABOLISM MECHANISMS

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**Keywords:** organic chemistry, analytical chemistry, internal standards, aroma

Many compounds of interest in wine are difficult to analyze since they are present in very small quantities or they are unstable. The need for reliable data led scientists to develop complex method in order to overcome the analytical difficulties and provide accurate quantitative data for grape or wine characterization.

For 10 years, we developed several chemical strategies to obtain analytical standards either as labelled analogues or as diastereomers to develop SIDA and DIDA analytical methods, respectively. These quantification methods afforded accurate and reliable results by suppressing analytical bias due to sample preparation. Several examples will be presented from deuterated analogues: varietal thiols [1], thiol precursors [2], Ochratoxin A [3], and diastereoisomers: Ochratoxin A [4] and hydroxycinnamic acids [5].

Another interesting application based on synthetic compounds lies in their possible exploitation as tracers. Indeed, the scale-up and optimization of chemical syntheses from µg to mg levels provided us with substantial amounts of molecules that could be used in metabolism studies. For example, we recently used labelled thiol precursors as tracers in Sauvignon Blanc musts for metabolism studies. Degradation of such tracers was monitored to highlight several key interconversion mechanisms and bring new elements in varietal thiol biogenesis knowledge [6]. In these applications, the choice of the labelling position (for Ochratoxin A for instance) or multilabelling possibilities (for thiol precursors) offer future opportunity to investigate detoxification process or to obtain insight in the metabolism of aroma precursors, respectively.

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**Analytical developments  
from grape to wine,  
spirits : omics,  
chemometrics approaches...**

## VI.0.1

# INTEGRATED MULTIBLOCK DATA ANALYSIS FOR IMPROVED UNDERSTANDING OF GRAPE MATURITY AND VINEYARD SITE CONTRIBUTIONS TO WINE COMPOSITION AND SENSORY DOMAINS

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**Keywords:** AMOPLS, sequential harvest, berry sugar accumulation, targeted metabolomics

Much research has sought to define the complex contribution of terroir (varieties x site x cultural practices) on wine composition. This investigation applied recent advances in chemometrics to determine relative contributions of vine growth, berry maturity and site mesoclimate to wine composition and sensory profiles of Shiraz and Cabernet Sauvignon for two vintages.

Grape maturation was monitored using a berry sugar accumulation model and wines made from sequentially harvested grapes at three stages for each variety and vintage. Comprehensive targeted grape analysis of amino acids, carotenoids, sugars, organic acids, anthocyanins and volatile compounds were combined with targeted wine volatile and non-volatile chemical measures of composition and sensory descriptive analysis. Chemometric models of balanced sample sets derived from the pool samples were used in an ANOVA multi-block framework with orthogonal projection to latent structures (Boccard and Rudaz, 2016) to elucidate the relative importance of model design factors.

Multiple data matrices derived from the experimental design factors are subtracted from the original data matrix to obtain pure effects and interaction submatrices with structured orthogonal data. A response matrix is derived from the positive eigenvalues associated with SVD of each effect matrix and residuals are then added to each submatrix prior to kernel OPLS. Model performance evaluated from residual structure ratio (RSR), goodness of fit ( $R^2Y$ ) and permutation testing identified the significant factors from each model. Projection of sample scores of significant factors against scores of the residual matrix is used to assess sample clusters with confidence intervals based on Hotelling  $T^2$ .

Loadings from significant experimental factors of each model were used for hierarchical cluster analysis (HCA) with Euclidean distance measures and Wards grouping criteria. Prior to HCA scores and loadings are rotated to consistent presentation of factor levels in model plots. A conservative interpretation of loadings heat maps was considered appropriate and a summary heat map for explanatory factors is presented that enable interpretation of the impact of cultivar, site (soil x mesoclimate), grape maturity and region on grape and wine composition. The integrated data driven approach used in this investigation may be of assistance for other investigators for omics based experiments.

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## VI.O.2

**CAN WINE COMPOSITION PREDICT QUALITY? A METABOLOMICS APPROACH TO ASSESSING PINOT NOIR WINE QUALITY AS RATED BY EXPERTS.****Emma SHERMAN**

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**Keywords:** Wine quality, Pinot noir, Metabolomics, Sensory

The perception of wine quality is determined by the assessment of multiple sensory stimuli, including aroma, taste, mouthfeel and visual aspects. With so many different parameters contributing to the overall perception of wine quality, it is important to consider the contribution of all metabolites in a wine when attempting to relate composition to quality. Presently, links between wine composition and quality are largely anecdotal, with winemakers relying on their experience, refined palates, and well established measures of wine quality such as alcohol content, phenolic composition and the absence of major faults to produce high quality wines.

In this study, we assessed relationships between wine composition and quality ratings determined by wine experts. Forty-eight Pinot noir wines from two vintages and several geographic regions around the world were subjected to sensory and chemical analysis. A panel of experts made up of wine industry professionals (n = 24) assessed the quality of the wines, as well as a number of other sensory attributes. The wines were analysed by untargeted reverse phase UHPLC-MS, and untargeted HS-SPME-GC-TOF-MS to obtain the non-volatile and volatile profiles of each wine respectively. Partial least squares regression of the non-volatile, volatile and combined chemical profiles, together with ratings of wine quality by experts, showed that the non-volatile profiles were more strongly correlated with perceived wine quality than the volatile profiles. Some new correlations between wine metabolites and quality ratings were found: several dipeptides and unsaturated fatty acids were positively associated with wine quality, and a volatile acetamide was strongly negatively correlated. Both the non-volatile wine matrix and the volatile profile of a wine should be considered in the relationship between Pinot noir wine composition and quality.

### VI.0.3

## D-WINES: USE OF LC-MS METABOLOMIC SPACE TO DISCRIMINATE ITALIAN MONO-VARIETAL RED WINES

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**Keywords:** mass spectrometry, wine authenticity, bioinformatics, metabolomics

Studying wine metabolome through multiple targeted methods is complicated and limitative; since grapes, yeasts, bacteria, oxygen, enological techniques and wine aging collaborate to deliver one of the richest metabolomic fingerprint. Therefore, untargeted metabolomics, that developed and evolved as a consequence of the need to obtain a comprehensive characterization of the organic molecules in any biological sample, is the current methodology offering the best coverage of wine metabolome. Taking into account the large genetic diversity, the diversity of the climate and of the agronomical practices, and the wide winemaking culture characterizing the Italian wines, the metabolomic untargeted approach appears as an appropriate analytical tool to study such metabolic space.

According to the national project D-Wines, 110 single-cultivar red wines from the 2016 vintage were collected directly from wineries across different regions of Italy: Sangiovese from Tuscany and Romagna, Nebbiolo from Piedmont, Aglianico from Campania, Nerello Mascalese from Sicily, Primitivo from Apulia, Raboso and Corvina from Veneto, Cannonau from Sardinia, Teroldego from Trentino, Sagrantino from Umbria, and Montepulciano from Abruzzo. The wines were analyzed according to a well-defined RP-UPLC-HRMS-QTOF-MS protocol.

The results of the data analysis, after their validation: a) confirmed untargeted LC-MS-based metabolomics as a powerful authenticity tool; b) provided indications about the similarity between the cultivars, clustering the wines in three major groups (Primitivo – Nebbiolo, Corvina, Raboso, Sangiovese – Teroldego, Sagrantino, Cannonau, Nerello, Aglianico, Montepulciano); c) furnished a rich list of putative markers characterizing each cultivar, where Primitivo, Teroldego and Nebbiolo had the maximum number of unique putative markers; d) revealed that the putative markers were not only phenolic metabolites; and e) pointed out rt/mz chromatographic sections helpful to distinguish each cultivar from the others.

This study, together with other D-Wines analytical results, is directed to understand the diversity of Italian red wines and to characterize them in term of metabolic space coverage/variability and taste and in consequence comprehend better their quality.

**Acknowledgements:** MIUR project N. 20157RN44Y. A. Curioni, A. Gambuti, V. Gerbi, S. Giacosa, G.P. Parpinello, D. Perenzoni, P. Piombino, A. Rinaldi, S. Río Segade, B. Simonato, G. Tornielli, S. Vincenzi

## VI.O.4

## LC-MS BASED METABOLOMICS AND TARGET ANALYSIS TO STUDY THE CHEMICAL EVOLUTION OF WINES STORED UNDER DIFFERENT REDOX CONDITIONS

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**Keywords:** Oxidation, Reduction, Metabolomics, VSCs

Oxygen is a key player in oenology, since its effects can be a blessing, benefiting wine quality, or a curse causing irreversible damage. Therefore, many modern winemaking choices tend to favor reduction, even if the severe lack of oxygen can be responsible for a loss in quality due to the formation of Volatile Sulfur Compounds (VSCs) able to cause aroma depreciation, such as H<sub>2</sub>S and MeSH. The aim of this study was to measure the changes caused to the metabolic space of several red and white wines stored under different levels of oxidative or reductive conditions.

Twelve wines (8 reds and 4 whites) were stored in strict anoxic conditions at 25°C (1, 2 and 3 months) and also at 35°C for 3 months. Aliquots of the same wines were also micro-oxygenated at 25°C during 3 months at different doses of oxygen. The redox potential of all samples was measured and then they were analyzed with an untargeted approach protocol by using a UPLC-HRMS-QTOF instrument to register their metabolic fingerprint; and with a targeted method by using a GC-SCD instrument to analyze the free and Brine Releasable (BR) forms of VSCs. A typical in-house workflow for the data analysis of the metabolic data was used for the quality control of the data-set and for the biomarker discovery and annotation.

The redox potential measurements indicated the reliability of the sample set, since as expected it increased in the presence of oxygen and decreased in anoxic conditions. The LC-MS untargeted analysis generated a dataset of over 10000 features, which after the statistical analysis our attention was focused to approximately 150 tentative markers. These markers were classified in four groups depending on their behaviour under the different conditions. Between the markers were annotated various anthocyanins, such as peonidin 3-glucoside-catechin which decreased under oxidative conditions but remained stable in reduced samples. By contrast, malvidin 3-glucoside decreased also under anoxic conditions although at lower rates with respect to oxidative conditions. Some sulfonated indoles were identified as markers of oxidation conditions. As far as the free VSCs, the highest concentrations were determined in the more reduced samples. The study provides a new understanding about the role of oxygen and of its absence in wine aging.

**Acknowledgements:** Work funded by the Spanish MCIU AGL2017-87373-C3-1R. I.O. received the grant (CB 8/18) from "Programa Ibercaja-CAI Estancias de Investigación" funded by Universidad de Zaragoza, Fundación Bancaria Ibercaja and Fundación CAI.

## VI.O.5

# FULLY AUTOMATED NON-TARGETED GC-MS DATA ANALYSIS

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**Keywords:** metabolomics, non-targeted, GC-MS, exploratory data analysis

Non-targeted analysis is applied in many different domains of analytical chemistry such as metabolomics, environmental and food analysis. In contrast to targeted analysis, non-targeted approaches take information of known and unknown compounds into account, are inherently more comprehensive and give a more holistic representation of the sample composition.

Besides chromatographic techniques coupled to high resolution mass spectrometry such as LC-HRMS, gas chromatography with unit resolution mass spectrometry is still regularly utilized for non-targeted profiling or fingerprinting. This is mainly due to high separation power of GC and a wide availability and low costs of quadrupole mass spectrometers.

Although several non-targeted approaches have been developed, data processing still remains a serious bottleneck. Baseline correction, feature detection, and retention time alignment can be prone to errors and time-consuming manual corrections are often necessary. We therefore developed an automated strategy to non-targeted GC-MS data avoiding feature detection and retention time alignment. The novel automated approach includes segmentation of chromatograms along the retention time axis, multiway decomposition of transformed segments followed by a supervised machine learning pipeline based on gradient boosted tree classification on the decomposed tensor [1, 2].

In order to make this novel data analysis strategy available to scientists without programming background, we developed a convenient browser based application. For the here presented interactive browser application the open source Python packages Bokeh and HoloViews were used. The application will be online freely available soon.

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## VI.O.6

## Q-NMR MEASUREMENTS : QUANTITATIVE ANALYSIS OF WINE COMPOSITION APPLIED TO BORDEAUX RED WINES AUTHENTICITY CONTROL

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**Keywords:** wine, authenticity, qNMR, multivariate statistics

Traceability of wine is today a consumer demand and a scientific challenge. The methods of analysis must be able to control three fundamental parameters: the geographical origin, the grape varieties, and the vintage. With these focus, the CIVB supports the creation of a VRAI platform (Wine-Research-Authenticity-Identity) within the ISVV (Institute of Vine and Wine Sciences). This platform aims to develop analytical tools to guarantee the origin of a wine. Quantitative Nuclear Magnetic Resonance (qNMR) may be a great tool to help authenticate wines. The acquisition of a large number of wine parameters requires a small volume (a few hundred microliters) and the analysis is performed in a few minutes. This innovative analytical technique can therefore be useful to characterize wines quality and authenticity particularly in the context of priceless wine.

A NMR-based metabolomics method was developed to semiautomatically quantify many wine components [1]. An original approach based on similarity score (s-score) was developed for wine comparison. Using this approach, a comparative evaluation of the results obtained for three sets of authentic high-valued wines and suspect wines was studied with two methodologies: (i) usual wine analysis, based on the use of multiple techniques, which is the traditional way of analysis for wine authentication and (ii) q-NMR profiling [2]. In order to consider a global aging uncertainty, samples from the same batch from old vintages were analyzed to estimate aging impact on wine composition. Results showed that q-NMR can detect cases of fraud by comparison with the original wine provided by the estate, according to conclusions of official methods.

More, a database of commercial French wines was built with q-NMR data to examine the specific Bordeaux red wines fingerprinting. Several statistical analyses were performed to classify wines according to their geographical origin, vintage. Results revealed a singular imprint of Bordeaux wines in comparison with other French wines, with classification rates ranging from 71% to 100%. These analyses highlighted several specific metabolites of Bordeaux red wines and showed the influence of terroir in the discrimination. Also, Bordeaux subdivisions were investigated, and effects of wines evolution during bottle aging and vintage were pointed out. These studies provide a global and practical description of the potential of q-NMR for wine authentication.

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## VI.0.7

# A NEW GRAPHICAL INTERFACE AS A TOOL TO INTEGRATE DATA FROM GC-MS AND UPLC-MS-QTOF: NEW COMPOUNDS RELATED WITH PORT WINE AGING.

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**Keywords:** data-fusion, Port, ageing, omics

Port wine value is related to its molecular profile resulting from the changes occurring during the ageing period. It is of empirical knowledge that the style is greatly affected by the oxidation regimens, i.e. bottle versus barrel storage. The final quality is rated based on sensory evaluation and the correspondent chemical profile remains largely unknown. This lack of knowledge and understanding significantly limits the ability to improve/drive Port Wine quality and consistency.

Unravelling the chemical changes, occurring during ageing, that are responsible for the wine flavour, constitutes a critical task when one attempts to address issues related to authenticity and sensory quality.

It has been demonstrated that some key odorants play a critical role in the perceived oxidized character of wines (1, 2). Nevertheless, the mechanisms for these key odorants formation are not fully understood; only that temperature and mainly oxygen have a synergistic impact on their formation (3). Recently it has been demonstrated that Strecker degradation substrates such as: phenolics, sugars and metals can interact resulting in a unpredictable formation of flavour molecules.

In order to have an holistic view of the chemical system a pipeline was developed based on UPLS-MS-QTOF and GC-MS data acquisition followed by data fusion. The process is hyphenated with an in-house peak picking interface, coupled with multi- and -univariate statistics to get the most relevant compounds related in this case with Ports stored from 1 to 150 years old.

In this work the "omics" interface was validated with a set of 37 wines; 42 biomarkers were extracted from GC-MS and 152 from UPLC-MS-QTOF.

The development of tools such as network reconstruction provided considerable amount of information that contributed to the understanding of the kinetic contexts of the molecules (through ageing time). Clusterization of volatiles and non-volatile compounds brought further new information regarding the interaction between mechanisms and new compounds were identified, such as: SO<sup>2</sup>-phenolics reactions, phenolics-phenolics, phenolics-aldehydes, amongst other.

This network-driven approach, integrating data from different equipment's. has proven to be an useful tool in identifying compounds of interest related to changes occurring during food storage or ageing processes, as well as in better understanding the drivers of quality and authenticity in the final product.

## VI.O.8

## MOLECULAR CHARACTERIZATION OF WINES NUCLEOPHILIC POTENTIAL BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY

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**Keywords:** LC-QToF-MS, Nucleophilic compounds, Untargeted analysis, White wines oxidative stability

The knowledge about the molecular fraction associated to white wines oxidative stability is still poorly understood. However, the role of S- N- congaing compounds, like glutathione (GSH) and other peptides, as a source of reductant in many oxidation reactions protecting against heavy metals toxicity, or lipids and polyphenols oxidation as ROS-scavenger is today very well established. GSH is also reported being an important antioxidant, reacting as nucleophile substance that conjugates straightforwardly with reactive electrophiles resulting in foods and beverages chemical oxidative stability. It has been shown that, GSH efficiency against wines sensory oxidative stability is related to wines antioxidant metabolome consisting of N- and S- containing compounds like amino acids, aromatic compounds and peptides. These compounds present a strong nucleophilic character and their reactivity with wines electrophiles such as oxidized polyphenols, suggests the formation of stable adducts presenting lower oxidative potential. We consider that the knowledge behind the chemical composition of wines antioxidant metabolome is a key factor to estimate wines aging potential.

In that respect, the present study introduces an original determination of the pool of nucleophilic compounds that can react with quinones in wine acidic conditions. One step derivatization of nucleophiles has been realized in wines with no pH adjustment by using 4-methyl-1,2-benzoquinone (Q) as a nucleophilic probe. LC-MS-QToF analysis of 92 white followed by Multivariate analysis (PLS-DA) and Wilcoxon test allowed to isolate up to 141 putative nucleophilic compounds. Only 20 of these compounds were detected without derivatization, showing an increase in detection level by quinone trapping, especially for thiols. Moreover, annotation using online database (Oligonet, Metlin and KEGG) as well as elementary formula determined by isotopic profile and MS<sup>2</sup> analysis allowed to show an important proportion of amino acids and peptides and to identify 4 compounds (GSH, Cys, homocysteine and Pro). The majority of the putative peptides can contain amino acids that are known to have antioxidant properties (Val, Leu, Ile, Pro, Trp, Cys and Met).

These results show that derivatization of wines using Q allows to enhance thiol detection levels and to determine a pool of untargeted nucleophilic compounds that can be part of wines antioxidant metabolome.

## VI.O.9

# INFLUENCE OF THE MALOLACTIC FERMENTATION ON WINE METABOLOMICS OR DRASTIC METABOLOMICS CHANGES DUE TO MALOLACTIC FERMENTATION

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**Keywords:** bacteria, malolactic fermentation, metabolomic, wine

It is well known that lactic acid bacteria modify the wine volatile compound. However, very few data are available regarding metabolite changes that occurred during the malolactic fermentation (MLF). In order to have a clearer picture of the metabolic signature of the bacteria in wine during the MLF, we have analyzed the exometabolome before and after MLF of wine fermented with 6 different yeast strains and 2 different bacteria. To this purpose, metabolomics analyses were carried out by LC-TOF-MS.

The PCA analyses of the metabolomics data clearly distinguish samples at the end of alcoholic fermentation from samples after malolactic fermentation and samples from co-inoculation. These results confirmed the impact of bacteria on wine metabolome but also underlined the fact that co-inoculation of bacteria with yeast in must does not result in the same wine than sequential inoculation, from a metabolite point of view. This result clearly indicates that both matrix (must or wine) and yeast bacteria interactions are responsible for the observed differences. A focus on the comparison of wine before and after malolactic fermentation conducted by the lactic acid bacteria VP41 revealed a clear cut difference between the wines as represented by PLS-DA. These results confirmed the drastic changes of the wines due to malolactic fermentation. Some of the compounds catabolised or synthesized by the bacteria during MLF allows to identify specific metabolic pathway involved during MLF such as for example glycosyl hydrolases, which convert flavonoid glycosides to the corresponding aglycones, and esterase, degrading methyl gallate, tannins, or phenolic acid ester.

## VI.O.10

## DIFFERENT STRATEGIES FOR THE RAPID DETECTION OF HAZE-FORMING PROTEINS (HFPS)

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**Keywords:** haze-forming proteins, biosensor, FT-IR, chemometric analysis

Over the last decades, wine analysis has become an important analytical field, with emphasis placed on the development of new methodologies for characterization and elaboration control. Advances in wine chemistry knowledge allow the relation of specific wine faults or defects to the compounds responsible for those unpleasant characteristics. In most cases, those compounds are already naturally present in wine, but their effect does only become noticeable when their concentration exceeds the "sensory threshold".

Among the different instabilities that can occur, protein haze formation is a serious quality defect because consumers perceive hazy wines as "spoiled" [1]. Protein haze is caused by aggregation of residual grape pathogenesis-related proteins, particularly, thaumatin-like proteins and chitinases upon exposure to elevated temperatures during storage or transportation. Unfortunately, a specific method for the detection, or treatment, of such proteins in affected wines does not exist, and current practice is to use fining agents such as bentonite for their removal. On the one side, this might have a negative impact on wine quality, as not only haze forming proteins (HFPS) are being removed, but also other compounds that do impact on wine flavour/aroma. On the other side, the lack of a specific method to quantify HFPS, tends to result in over-fining, which in turn has a more detrimental impact in wine quality, fining cost and waste generation.

Herein we investigate on the development of an easy-to-use sensory device that allows to detect the presence of HFPS. To this aim, three different approaches have been explored. On the one hand, two different impedimetric biosensors based on screen-printed electrodes were developed, and their performance assessed towards standard solutions as well as wine samples. As an alternative, Fourier Transform Infrared (FT-IR) spectra were collected for different wine samples and chemometric tools such as discrete wavelet transform (DWT) and artificial neural networks (ANNs) were used to achieve the quantification of HFPS proteins. Detection of HFPS at the µg/L level has been achieved with both impedimetric biosensors in standard solutions, whereas the FT-IR-based approach allowed their quantification at the mg/L level in wine samples directly.

[1] S.C. Van Sluyter, *et al.* J. Agr. Food Chem., 63 (2015) 4020-4030.



**Sensory properties,  
psychophysics,  
experimental economy,  
connections with  
neurosciences**

## VII.O.1

# COMPARING THE EFFECTS OF VISION, SMELL AND TASTE IN RED WINE QUALITY JUDGMENTS BY EXPERTS: SENSORY CUES, MENTAL IMAGERY AND VERBAL REPRESENTATIONS AS DRIVERS OF CONSENSUS IN THE MULTISENSORY SPACE

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**Keywords:** Wine tasting, Perceptual mechanisms, Mental Imagery, Vocabulary

In this study, we evaluated the contributions of vision, smell and taste to red wine quality judgments by expert wine tasters. Whereas previous studies specified the modulating effects of gustatory traits [1], culture and expertise [2, 3], our objective was to gain a better understanding of the perceptual mechanisms, with special consideration of the psychological representations that predict consensus in red wine quality judgments. To this aim, we compared wine tasters' responses in unconstrained (i.e., all senses involved) and constrained wine tastings (i.e., unisensory: "visual", "smell" and "taste"; multisensory: "visual-smell", "visual-taste" and "taste-smell") over six wine tasting sessions. In each session, wine tasters rated the quality of 20 red wines from a Protected Designation of Origin (PDO, premium vs. secondary wines), starting with an unconstrained tasting and then followed by a constrained tasting. We also collated predictors based on wine tasters' responses to self-report questionnaires that assessed vividness of mental imagery in visual, smell, taste, somesthetic and wine contexts. Using a series of vocabulary tasks, we also evaluated whether lexical capacity predicts consensus in red wine quality judgments.

Overall, our results showed a coherent quality concept across unconstrained and constrained wine tastings, with a clear quality distinction favoring premium wines. However, principal component analyses suggested a better quality judgement consensus with unisensory vision cues compared to all other sensory conditions. Going further, regression analyses also revealed specific drivers of red wine quality judgment consensus that are based on age, vividness of wine mental imagery, lexical capacity and consensus, as well as unisensory smell consensus and to a lesser degree, multisensory visual-taste consensus and unisensory taste consensus.

Common experiences with wine, as well as the number of years tasting might promote strong vividness for wine representations (images and vocabulary), which in turn help predict wine tasters' inclusion to the consensus involved with red wine quality judgments. Taken together, this study gives us an insightful look at the individual knowledge base, as well as the experience and representational cues that could delineate expert status. Further research in this direction could help promote informed teaching curricula in professional training and expert wine tasting.

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**VII.O.2****DOES WINE EXPERTISE INFLUENCE SEMANTIC CATEGORIZATION OF WINE ODORS?****Léa KOENIG**Cécile Coulon-Leroy<sup>1</sup>, Ronan Symoneaux<sup>1</sup>, Véronique Cariou<sup>2</sup>, Evelyne Vigneau<sup>2</sup>

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**Keywords:** expertise, odor categorization, free sorting, additive tree

Aromatic characterization is a key issue to enhance wines knowledge. While several studies argue the importance of wine expertise in the ability of performing odor-related sensory tasks, there is still little attention paid to the influence of expertise on the semantic representation of wine odors. This study aims at exploring the influence of subject's expertise on the semantic space of wine's odor.

156 subjects were recruited (72% consumers of wine and 28% professionals from viticulture sector). Subject level of expertise was measured by means of a questionnaire encompassing three criteria: product experience, subjective knowledge and objective knowledge. Four groups of subjects were identified using Rasch model corresponding to four levels of expertise: novices, intermediates, connoisseurs and experts. Thereafter, subjects performed a sorting task on 96 labels of odors and add a title to the groups. To investigate the influence of subject's expertise on the semantic space of wine's odor, the four groups' clusters were compared on several criteria: number and size of odor groups from the sorting task and agreement between the subjects within each cluster. Dissimilarity matrices were also compared to highlight differences between clustering. Finally, to represent the semantic odor space, additive similarity trees were performed on sorting data.

Results show that number and size of odor groups are likely to be the same between the four clusters (between 26 and 31 groups in average and 3 odors per group in average for the four clusters) and no differences of agreement within each cluster can be highlighted. Additive trees performed on clusters show that most of the branches are the same between the two clusters: fruity, floral, woody, vegetal, spicy, etc. Overall, semantic representation of odors is consensual regardless the level of expertise. But, some differences may be underscored. These latter ones are mostly between expert's cluster and the three other clusters.

This work highlights that subjects, professionals or not, have the same structuration of wine odor attributes: they categorize odors according to the odorant source. However, some attributes do not have the same meaning for experts and non-experts which lead to a different categorization. This study is the first step toward a sensory tool for wine characterization aiming at simplifying and standardizing the process of describing wine odors, from generic to more specific attributes.

### VII.O.3

## EXPLORING MULTISENSORY INTERACTIONS THROUGH THE STUDY OF ASTRINGENCY DIVERSITY OF MONO-VARIETAL ITALIAN RED WINES

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**Keywords:** mouthfeel and odor, diversity, interactions, chemometrics

According to the OIV Focus 2017 estimating the vine varieties distribution in the world, Italy is the richest grape producing country in terms of varieties. This rich biodiversity translates into a wide sensory diversity of the wines that was never systematically investigated. The D-Wines (Diversity of Italian Wines) project, is aimed to start filling this gap by getting a wide chemical and sensorial multi-parametric dataset about 11 mono-varietal red wines (Aglianico, Cannonau, Corvina, Montepulciano, Nebbiolo, Nerello Mascalese, Primitivo, Raboso, Sagrantino, Sangiovese, Teroldego) representative of the Italian territory and by focusing on tannins and astringency.

In this frame, the astringency diversity of a set of 112 wines belonging to the 11 varieties, was investigated by sensory analysis adopting a multi-steps analytical strategy. A first experiment by sorting, allowed to reduce (AHC analysis) the sample-set to 77 wines, representative of the intra-varietal similarities and diversities in terms of astringency sub-qualities. A second experiment by descriptive analysis was performed on the selected wines and allowed to obtain their sensory profiles (astringency, taste, odor). Both intra- and inter-varietal significant differences of each sensory variable was tested by ANOVA ( $p < 0.05$ ). Quantitative data concerning astringency were analyzed through Discriminant Analysis (DA).

Results showed that the 6 variables describing astringency (drying, harsh, unripe, dynamic, complex, surface smoothness; Gawel *et al.*, 2000) allowed a good discrimination ( $F1+F2$ : 78%) of the wines according to the grape variety. Factor scores of each sample allowed their reclassification into the variety for which the probability of belonging was the greatest. The 57% of the wines resulted correctly reclassified, with Nebbiolo showing the highest value (83%) and Nerello Mascalese the lowest (0%).

The quantitative data concerning the well reclassified wines were used to develop "Astringency spectra", models representing the astringency features of each mono-varietal wine.

These "Spectra" were compared to those of the corresponding deodorized wines in order to investigate the multisensory interactions between astringency, taste and odor variables. Several significant correlations were detected (e.g.  $R^2 > 0.5$ : drying and dynamic, drying and dehydrated fruit, complex and spicy were positively correlated while harsh and acid were negatively correlated).

*Acknowledgements: MIUR project N. 20157RN44Y. Other components of D-Wines project: P. Arapitsas<sup>4</sup>, A. Gambuti<sup>1</sup>, S. Giacosa<sup>6</sup>, M. Marangon<sup>3</sup>, A. Ricci<sup>7</sup>, L. Rolle<sup>6</sup>, S. Rio Segade<sup>6</sup>, B. Simonato<sup>3</sup>, G. Tornielli<sup>2</sup>, A. Versari<sup>7</sup>, S. Vincenzi<sup>3</sup>.*

## VII.O.4

### RED WINE ASTRINGENCY: EVOLUTION OF TRIBOLOGICAL PARAMETERS DURING DIFFERENT HARVEST DATES

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**Keywords:** wine astringency, tribology, human saliva, harvest dates

Astringency is a specific oral sensation dominated by dryness and puckering feeling and is one of the leading quality factors for red wines, as well as some fruit products. Based on this sensory parameter, are made relevant decisions in wine production including the moment of grape harvest (phenolic ripeness), the time and intensity of maceration, the time and type of aging process, and the target market of wines.

Notably, the selection of the optimal grape astringency during ripeness is one of the most crucial decisions in winemaking. However, grape astringency is an attribute challenging to evaluate and standardize by tasters since the grapes are heterogeneous and generate along their ripeness different sensory descriptors, such as the typical drying astringency found in immature grapes. Here we used a tribological system to determine the red wine astringency produced on different harvest dates. Mixtures of whole human saliva and red wines as Cabernet Sauvignon and Carménère, with similar tannin content but different sub-quality (rough and soft/velvety, respectively), were evaluated by their lubrication behavior.

Red wines produced significant changes in the saliva friction coefficient during the harvest dates, with an opposite evolution between the Cabernet Sauvignon and Carménère.

Also, microstructure observation revealed differences between conformation and surface of the tannin-protein aggregates of both red wines, suggesting a correlation between them and the astringency sensory perception. Results from this work demonstrate that tribology techniques can be a useful tool for both to evaluate astringency on red wines and to help us to understand the phenomenon of sub-qualities.

## VII.O.5

# SENSORY EVALUATION OF GRAPE BERRIES: PREDICTIVE POWER FOR SENSORY PROPERTIES OF SAUVIGNON BLANC, RIESLING AND PINOT NOIR WINES

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**Keywords:** Sensory evaluation, grape berry, grape maturity, wine

Sensory analysis of grape berries is a common tool to evaluate the degree of grape maturation and to make sound picking decisions. However, most of it is based on anecdotal knowledge and scientific studies relating berry and wine properties are rather limited [1].

Ten grapes of each variety (Sauvignon Blanc, Riesling, Pinot Noir) were picked weekly. Berries were dissected manually to obtain berries with intact peduncles. Using sucrose solutions of different densities, berries were separated into three density fractions of 1.070, 1.080, and 1.090. Three individual berries were assessed of each density group on each picking date. White and red wines were made from grapes picked concurrently with berry samples and were fermented in duplicates [2].

For Sauvignon Blanc 13 out of 21 visual, haptic, odor and taste attributes varied significantly among the three picking dates. Firmness and yellow color of the berries and brown color of the seeds and bitter berry skins yielded the largest F-ratios. Green notes in pulp and skin decreased during ripening. Variation of grape berry density yielded 14 significant attributes, including sweet and sour taste as well as fruity perception [2].

In a PCA the first PC was governed by ripe versus unripe attributes, while PC2 was dominated by presence versus absence of green odors in pulp and skin. Sensory evaluation revealed better grouping by density than grouping by picking date.

Correlating berry and wine sensory brown seeds and sweet pulp correlated with increased peach and passionfruit notes in the wines. However, no correlation was found for green notes depicted in berries and green bell pepper nuances in the wines or fruity aspects in the berry and passion fruit / peach intensities in the wine.

In conclusion, berry sensory yields a good characterization of the ripening process as well as technological grape properties, but is rather limited in the prediction of wine sensory properties.

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**VII.O.6****CAN VARIETAL 'APRICOT' AROMA OF VIOGNIER WINE BE CONTROLLED WITH CLONAL SELECTION AND HARVEST TIMING?****Tracey SIEBERT**

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Recent wine-like reconstitution sensory studies confirmed that several monoterpenes were the key aroma compounds in the perception of an 'apricot' aroma attribute in Viognier wine. Other aroma compounds, including a set of aldehydes and several  $\gamma$ -lactones, were also indicated to be related to 'apricot' aroma in that study, but the addition of these compounds to the reconstitution gave ambiguous results.

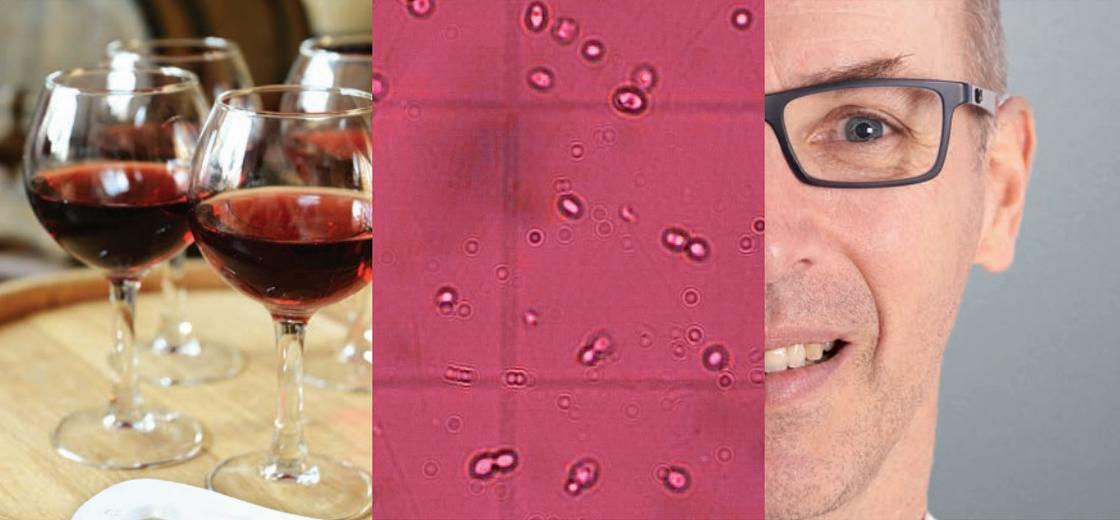
To investigate these interactions, further reconstitution sensory studies were conducted. Firstly, in a wine-like model matrix, the aldehydes were found to suppress 'apricot' aroma intensity, while  $\gamma$ -lactones significantly enhanced the intensity of 'apricot', but only in the presence of a higher concentration of monoterpenes. Secondly, a neutral Chardonnay wine base spiked with the monoterpenes and  $\gamma$ -lactones together, or with only the monoterpenes added, was considered to have a similar 'apricot' aroma to a typical Viognier wine, whereas if spiked with only  $\gamma$ -lactones, then its aroma was not similar. Finally, a sensory difference study was conducted by comparing single or double strength  $\gamma$ -lactones in Chardonnay wine with added monoterpenes. No significant difference was found between the monoterpene-spiked Chardonnay wine and when  $\gamma$ -lactones were also added. Thus,  $\gamma$ -lactones are unlikely to impart or enhance 'apricot' aromas in white wine.

Monoterpenes are grape-derived aroma compounds, but little is known regarding their accumulation in Viognier grapes. Having established the importance of monoterpenes to the perception of varietal 'apricot' aroma in Viognier wines, it is likely that controlling their concentration in the grapes can influence the 'apricot' aroma intensity in the resultant wine.

To establish if clonal selection and harvest timing could be used as tools to modulate 'apricot' aromas in Viognier wines, vineyard studies were conducted. Eleven Viognier clones were assessed over three vintages. Large differences were found in the concentration of the monoterpenes between the clones. In a further study of four Viognier clones, two clones showed similar monoterpene concentration profiles throughout ripening, but the other clones were substantially lower in monoterpene concentration. Subsequently, a winemaking study was carried out to assess the effect of clone and grape ripeness on 'apricot' character in Viognier. Grapes from two Viognier clones were both picked at two ripening timepoints and from two wine regions with different climates.



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# Short communications (Plenary, Workshop)

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# Plant and Environment, Grape quality

## I.SC.1

# INFLUENCE OF PRE-HARVEST ELICITORS TREATMENT DURING RIPENING PERIOD ON PHENOLIC COMPOSITION IN MONASTRELL GRAPES

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**Keywords:** Elicitors, Monastrell, Phenolic compounds, Grape

Phenolic compounds are very important in crop plants, which is why there have been the subject of a large number of studies. There are three main reasons for optimising the level of phenolic compounds in crop plants: their physiological role in the plants, their technological significance for food processing, and their nutritional characteristics.

Several techniques have been applied to improve the phenolic content of grapes. One such strategy developed in recent years is to apply elicitors, molecules that are able to trigger plant defence responses, thus contributing to plant resistance against pathogen attacks, and to activate secondary pathways, resulting in the accumulation of phenolic and aromatic compounds. In agricultural practice, elicitor treatment may represent an effective alternative to conventional agrochemicals [1].

In recent years, elicitor treatments of several grape varieties and consequent modifications in the corresponding grape and wine composition have been reported. For example, the pre-harvest application of BTH to Monastrell grapes led to increase levels of phenolic compounds in the treated grapes and the corresponding wines. [1]. It can be said that elicitation is a good strategy for inducing the synthesis of different classes of bioactive secondary metabolites, although the efficacy of such treatment mainly depends on individual plant genetics, the nature of the elicitor and the dose used.

Our research group has focused its interest on the effect of the pre-harvest application (at veraison) of two elicitors: benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and methyl jasmonate (MeJ), and a mixture of both on the phenolic composition during ripening period in two consecutive seasons (2016 and 2017); in order to be assessed whether veraison is the best moment for applying the elicitor or whether the application date could be optimised to obtain a maximum phenolic contents at harvest.

The results were different in function of the year study, obtaining higher concentrations of phenolic compounds in 2016 compared to 2017. On the other hand, the treatments showed higher results in all phenolic compounds studied excepted in the stilbenes when there were compared to the control grapes. Finally, some of the phenolic compounds analyzed obtained higher concentrations before harvest date. Therefore, our future objective will be to optimise the moment of elicitor application in order to obtain their maximum effect at the moment of harvest, probably by delaying the application until some weeks after veraison.

[1] Ruiz-García, Y. et al. *Journal of Agricultural and Food Chemistry*, 2012, 60, 1283.

## I.SC.2

## COMING OF AGE: DO OLD VINES ACTUALLY PRODUCE BERRIES WITH HIGHER ENOLOGICAL POTENTIAL THAN YOUNG VINES? A CASE STUDY ON THE RIESLING CULTIVAR.

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Consumers and the wine industry tend to agree on the ability of old vines to produce fruit that allows the production of wine of superior character. However, despite past and ongoing research, objective evidence of this point of view is still debated and studies on robust, specifically dedicated plots are scarce.

Thus the impact of grapevine age on berry oenological potential and wine quality remains an open question. To try to objectively address the issue, a unique vineyard was established at Geisenheim University, Germany. It was planted in 1971 with cv. Riesling grafted on 5C Teleki. In 1995 and 2012, several rows were uprooted and replanted with the same rootstock/scion combination, resulting in a vineyard with alternate rows of identical plant material, but with different planting dates. The parameters of technical maturity and grape composition at harvest were analyzed during seasons 2014, 2015, 2016 and 2017 combining HPLC and enzymatic methods. Separate micro-vinifications were made for each age group and wine composition was analyzed by a combination of 1H-NMR and SPE-GC-MS.

The results showed that technical maturity parameters (TSS, TA, must pH) were not significantly different across the three grapevine age groups. Berry composition showed little differences in quality-relevant metabolite contents, with the noticeable exceptions of  $\alpha$ -amino acid, skin flavonols, free and bound monoterpene and norisoprenoid amounts that were significantly higher in vines planted in 2012 compared to older vines, but only in seasons 2014 and 2015 where soil management practices of the young vines differed. Berries from vines planted in 1971 and 1995 did not exhibit significant differences in berry composition, throughout the four seasons. Wine chemical analysis complemented and confirmed results obtained in berry composition. Wine made from grapes produced by vines planted in 2012 had higher terpene and norisoprenoid amounts, compared to wines made with grapes from the two other vines groups, but only for the 2014 and 2015 vintages. Wines from grapes produced by vines planted in 1971 and 1995 did not exhibit significant differences in composition throughout the four seasons.

In conclusion, even though vines planted in 2012 exhibits significant differences in berry and wine composition for their two first vintages compared to older ones, these differences appeared to vanished once the vines were fully established and soil management practices became uniform.

*Acknowledgments:* This work was supported by a PhD grant to K. Bou Nader from the Forschungsring Deutscher Weinbau.

### I.SC.3

## NITROGEN STATUS OF VINES INFLUENCES AGED WINES AROMAS. EXAMPLES OF AGED CHAMPAGNE RESERVE WINES AND RED BORDEAUX WINES.

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**Keywords :** Ageing, Nitrogen status , Aromas, vines

The sensory definition of the aging bouquet of red Bordeaux wines has been shown to be structured around seven main aromatic nuances: "undergrowth", "spicy" "truffle", "fresh red- and black-berry fruits", "liquorice", "mint", and "toasted".<sup>1</sup> Some of these descriptors are also used to describe the aromatic notes of old Champagnes<sup>2</sup> suggesting common volatile compounds between these two types of wine.

The wine quality is closely related to its aromatic expression, influenced by the grape variety, viticultural management techniques and environmental factors, such as soil and climate.<sup>3</sup> It has been shown that the soil influences the taste of wines and the typicity of organoleptic expression. This is largely mediated by the availability of water and nitrogen.<sup>4</sup> The climate effect is mediated by air temperature and water balance.<sup>5</sup>

The volatile compounds developed during wine aging and involved in the expression of the bouquet may be affected by vine water and nitrogen status.<sup>6</sup> High nitrogen status in vines favors high nitrogen levels in both grape berries and wine. Thus, compounds such as tabanone, DMS, esters and aromatic heterocycles were measured in aged Bordeaux and Champagne reserve wines. Their concentrations were correlated to the water and nitrogen status in vine (evaluated during the season for the year production with Bordeaux vine) and the amino acids concentration in wines for Champagne reserve wine.

For both wines types, it has been revealed that the vine nitrogen status and the wine nitrogen composition have an important role on DMS, ester and aromatic heterocycles formation. Furthermore, a correlation between tabanones concentrations and the vine water status was observed.

Data collection for both red Bordeaux and Champagne reserve wines, which bouquet is well disguised, highlighted that nitrogen is involved in the various stages, common and/or different for both wine type, from vine formation to aged wine.

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#### I.SC.4

### WINE TANNINS : WHAT PLACE FOR GRAPE SEED?

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**Keywords:** Grape Maturity, Tannins, Extraction, Seed

Phenolic compounds are among the most important quality factors of wines. They contribute to the organoleptic characteristics of wine such as colour, astringency, and bitterness. Although tannins found in wine can come from microbial and oak sources, the main sources of polyphenols are skin and seed from grapes. Yet, the link between grape seed phenolic content and wine composition, or even the link between seed maturity stage and wine composition are poorly studied. This work describes and explains the seed tannins kinetics release in wine, but also the impact of seed maturity stage on seed tannins extractability.

The polyphenol content and composition of seeds at three different grape maturity stages were characterized (fifteen days before harvest, harvest and fifteen days after harvest). After that, an original approach of nanovinification was conducted. At each maturity stages three winemaking modalities have been produced in duplicate: (i) a control modality, (ii) a seed modality made of exclusively with seed and (iii) a skin modality made of exclusively with skin. The evolution of seed tannins release and tannins wine content has been followed during the winemaking, from alcoholic fermentation to maceration.

Independently from the grape maturity stage, skin tannins are present at the first step of winemaking contrarily to seed tannins presence which is progressive all along the vinification. The results indicated that (+)-catechin is the less extractable free flavan-3-ols compared to (-)-epicatechin and (-)-epicatechin gallate. Furthermore the mean degree of polymerization of seed proanthocyanidins seems to be directly linked to their extractability, raising the question of the impact of tannins interaction and cellular location on tannins extractability.



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# Grape and wine microorganisms : diversity and adaptation

## II.WS 1

# INFLUENCE OF MIXED FERMENTATIONS WITH *STARMERELLA BACILLARIS* AND *SACCHAROMYCES CEREVISIAE* ON MALOLACTIC FERMENTATION BY *LACTOBACILLUS PLANTARUM* AND *OENOCOCCUS OENI* IN WINES

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**Keywords:** *Starmarella bacillaris*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Oenococcus oeni*

Over the last years, the potential use of non-*Saccharomyces* yeasts to modulate the production of target metabolites of oenological interest has been well recognized. Among non-*Saccharomyces* yeasts, *Starmarella bacillaris* (synonym *Candida zemplinina*) is considered one of the most promising species to satisfy modern market and consumers preferences due to its peculiar characteristic (enhance glycerol and total acidity contents and reduce ethanol production). Mixed fermentations using *Starm. bacillaris* and *Saccharomyces cerevisiae* starter cultures represent a way to modulate metabolites of enological interest, taking advantage of the phenotypic specificities of the former and the ability of the latter to complete the alcoholic fermentation. However, the consumption of nutrients by these species and their produced metabolites may inhibit or stimulate the growth (and malolactic activity) of lactic acid bacteria (LAB). Consequently, a comprehensive understanding of the interactions between yeasts and LAB would be valuable for an efficient implementation of malolactic fermentation (MLF). To this end, the present study was carried out to elucidate the impact of this inoculation protocol on the growth and malolactic activity of *Lactobacillus plantarum* and *Oenococcus oeni* strains used to induce MLF, and finally on the chemical and volatile profile of Nebbiolo wines. MLF was carried out by inoculating LAB at the beginning and at the end of the alcoholic fermentation. Yeast inoculation protocol and the combination of tested species influenced LAB population dynamics and malic acid consumption. MLF in which *L. plantarum* was inoculated at the beginning of the fermentation were completed faster than those inoculated with *O. oeni*. On the contrary, when *L. plantarum* was inoculated at the end of alcoholic fermentation a stuck MLF was observed, while *O. oeni* completed successfully MLF, indicating that inoculation timing of both LAB species was critical to how rapidly starts and finish the MLF. The presence of *Starm. bacillaris* in mixed fermentations promoted *O. oeni* growth and increased malic acid consumption rate. Analysis from volatile composition showed that LAB species selection had a greater impact to aroma profile of the wines than inoculation time. This knowledge could be useful to better control MLF in mixed fermentations with *Starm. bacillaris* and *S. cerevisiae*, and underlines the importance of the inoculated yeasts on the growth and malolactic activity of the LAB.

**II.WS 2****MAY LACTIC ACID BACTERIA PLAY AN IMPORTANT ROLE IN SPARKLING WINE ELABORATION?****Maria DIMOPOULOU (1,2)**

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**Keywords:** microbial terroir, sparkling wine, lactic acid bacteria, genetic diversity

The elaboration of sparkling wine is a demanding process requiring technical as well as scientific skills. Uncovering the role of the terroir to the final product quality is of great importance for the wine market.

Although the impact of the yeast strains and their metabolites on the final product quality is well documented, the action of bacteria still remains unknown. The malolactic fermentation (MLF) is carried out by the lactic acid bacteria after the alcoholic fermentation in order to ensure the microbial stability during the second fermentation that takes place in the bottle or in tanks. *Oenococcus oeni* is the only selected species to drive MLF that has been commercialized for sparkling wine elaboration and it is naturally present on grapes, in the cellar and also in the final product. However, whether the bacterial strain contributes to the sensory characteristics of sparkling wine is still questioned. The present work focuses on the population diversity of lactic acid bacteria isolated from two sparkling wine production regions: the famous Champagne in France and the rising region of Amyntaion in North of Greece. The molecular typing method of multiple loci VNTR analysis was used to type the bacterial strains based on five tandem repeats loci was used in the present work. According to our results the bacterial strains isolated from sparkling wine production regions are usually differentiated from the rest by forming distinct genetic subgroups. The adaptation mechanism of the species to the particular conditions of sparkling wine is also reflected at phenotypic level. This observed phenotype can confer selective advantages to the bacteria in such acidic environments as these wines, with potential effects on sparkling wine foamability.

## II.WS 3

# NEW ANTIBACTERIAL PEPTIDES PRODUCED BY *SACCHAROMYCES CEREVISIAE* RESPONSIBLE FOR THE INHIBITION OF MALOLACTIC FERMENTATION

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**Keywords:** antibacterial yeast peptides, Wtm2p, Utr2p, GAPDH

In winemaking, several antimicrobial peptides (AMPs) produced by different strains of *Saccharomyces cerevisiae* were found to be responsible for the inhibition of malolactic fermentation (MLF) carried out by some strains of *Oenococcus oeni*. However, only two AMPs produced by one of the yeast strains studied were totally identified and their mechanism of action was described. In an attempt to identify new AMPs, a 5-10 kDa peptidic fraction produced by an oenological strain of *S. cerevisiae* and previously shown to strongly inhibit MLF carried out by a strain of *O. oeni* was further purified.

A synthetic grape juice medium fermented by the yeast strain was fractionated by ammonium sulfate precipitation combined with ultrafiltration. The 5-10 kDa peptidic fractions obtained at saturation degrees of 0%-20%, 20%-40% and 40%-60%, inhibited only the growth of *O. oeni* *in vivo* but not its ability to consume L-malic acid. The 5-10 kDa peptidic fraction recovered at a saturation degree of 60%-80% was the only one that inhibited both the bacterial growth and the malate consumption. It also inhibited the malolactic enzyme activity *in vitro* at a pH range between 3.5 and 6.7 in a cell-free enzymatic extract prepared from the same bacterial strain. Therefore, it was further purified by both anion and cation exchange chromatography. The eluates that inhibited the malolactic enzyme activity *in vitro* at the same pH range were migrated on Tricine SDS-PAGE and the protein bands were excised and sequenced by LC-MS/MS.

The sequencing revealed nine peptides originating from eight proteins of *S. cerevisiae* that play diverse vital roles in yeast cells. Two GAPDH cationic fragments of 0.9 and 1.373 kDa having a pI of 10.5 and 11 respectively, Wtm2p and Utr2p anionic fragments of 2.42 kDa with a pI of 3.5 and 4 respectively were considered to contribute the most to the MLF inhibition. However, it is likely that one or more of the nine peptides have worked synergistically to inhibit MLF. *In vivo*, they are supposed to enter the bacterial cytoplasm and inhibit the malolactic enzyme by mechanisms yet to be identified.

These results suggest that the 5-10 kDa fraction recovered at a saturation degree of 60%-80% contained at least two categories of peptides; the ones responsible for the bacterial growth inhibition and those responsible for the malate consumption inhibition. Whereas the fractions recovered between 0% and 60% contained only peptides that inhibited the bacterial growth.

**II.WS 4****EXTRACELLULAR SUBSTANCES OF LACTIC ACID BACTERIA INTERESTS IN BIOTECHNOLOGICAL PRACTICES APPLIED TO ENOLOGY****Peio ELICHIRY-ORTIZ**

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21078 DIJON CEDEX**Keywords:** extracellular substances, lactic acid bacteria, chemical characterization, enological practices

Extracellular substances (ECS) represent all molecules outside the cytoplasmic membrane, which are not directly anchored to the cell wall of microorganisms living through a planktonic or biofilm phenotype. They are the high-biomolecular-weight secretions from microorganisms (i.e. extracellular polymeric substances – EPS – proteins, polysaccharides, humic acid, nucleic acid), and the products of cellular lysis and hydrolysis of macromolecules. In addition, some high- and low-molecular-weight organic and inorganic matters from environment can also be adsorbed to the EPS. All can be firmly bound to the cell surface, associated with the EPS matrix of biofilm, or released as being freely diffusing throughout the medium.

In food industry, LAB are commonly studied and used because they can metabolize a wide variety of chemical entities (e.g. acids, carbohydrates...) determining the final product quality and stability. In wine, different LAB species have been identified. Among them, *Oenococcus oeni* and *Lactobacillus plantarum* are the two most encountered species and can subsist in wine environments, particularly in barrels in the form of biofilm phenotype. They possibly modify transfers of chemical compounds of interest at the wood/wine interface or actively influence them according to the enological practices adopted by the winemaker. To control and improve the use of this microbiological flora, it is essential to understand growth dynamics throughout time, particularly by persisting as a biofilm from one vintage to another.

Up to now, it is still not clear about the ECS composition in wine systems and how they act.

Combining different characterization measurements (e.g. mass yields, ATR-FTIR, SEC, LC-MS/MS...) will allow us to determine the role of these ECS during bacterial growth in function of physiological states (planktonic, biofilm) aiming to a better biotechnological control of these bacteria under novel enological practices.

Physicochemical analyses of the ECS produced by the model *Lactobacillus plantarum* WCFS1 strain in planktonic and biofilm conditions enable to determine the optimum growing phase for proteinaceous material production by varying growing media (i.e. 3 physicochemical semi-defined media and white grape must). ECS chemical composition unveils the presence of glycosidic enzymes from the same families for the 3 different semi-defined media.

## II.WS 5

# APPLICATION OF HIGH-THROUGHPUT SEQUENCING TOOLS FOR CHARACTERIZATION OF MICROBIAL COMMUNITIES DURING ALCOHOLIC FERMENTATION

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**Keywords:** Riesling, Metabarcoding, Metagenomics, DNA extraction

Developments in high-throughput sequencing (HTS) technologies allow us to obtain large amounts of microbial information from wine and must samples. Thus approaches, that are aimed at characterising the microbial diversity during fermentation, can be enhanced, or possibly even replaced, with HTS-based metabarcoding. To reduce experimental biases and increase data reproducibility, we compared 3 DNA extraction methods by evaluating differences in the fungal diversity with Riesling alcoholic fermentation samples at four different vineyards. The fungal diversity profiling was done using the genetic markers ITS2 and D2 using metabarcoding. The extraction methods compared consisted of a commercial kit, a recently published protocol that includes a DNA enhancer, and a protocol based on a buffer containing common inhibitor removal reagents. All methods were able to distinguish vineyard effects on the fungal diversity, but the results differed quantitatively.

From the results of extraction methods, we applied the chosen methods and further combined the HTS tools of metabarcoding and metagenomics, to characterise how microbial communities of those samples, and their subsequent spontaneously fermented derivatives, vary. We specifically explored microbial community variation related to vineyard level, and during alcoholic fermentation. The vineyard was shown to be strongly influencing the microbial communities. Functional analyses were additionally included to investigate the microbial interactions. An increase in non-Saccharomycetaceae fungal functions and a decrease in bacterial functions were also observed during the early fermentation stage. Overall, our results highlight the importance of standardizing DNA extraction methods when characterising fungal diversity from wine and related samples, and showcase how metagenomic functional analysis offer possibilities to improve our insights into the wine alcoholic fermentation process, including highlighting microbe interactions.

**II.WS 6****FLOR YEAST DIVERSITY AND DYNAMICS IN BIOLOGICALLY AGED WINES****Hervé ALEXANDRE**

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**Keywords:** flor yeast, FLO11, *Saccharomyces cerevisiae*, Vin Jaune

Wine biological aging is characterized by the development of yeast strains that form a biofilm on the wine surface after alcoholic fermentation. These yeasts, known as flor yeasts, form a velum that protects the wine from oxidation during aging. Thirty-nine velums aged from 1 to 6 years were sampled from "Vin jaune" from two different cellars.

We show for the first time that these velums possess various aspects in term of color and surface aspects. Surprisingly, the heterogeneous velums are mostly composed of one species, *S. cerevisiae*. Scanning electron microscope observations of these velums revealed unprecedented biofilm structures and various yeast morphologies formed by the sole *S. cerevisiae* species. Our results highlight that different strains of *Saccharomyces* are present in these velums. Unexpectedly, in the same velum, flor yeast strain succession occurred during aging, supporting the assumption that environmental changes are responsible for these shifts. Despite numerous sample wine analyses, very few flor yeasts could be isolated from wine following alcoholic fermentation, suggesting that flor yeast development results from the colonization of yeast present in the aging cellar. We analyzed the *FLO11* and *ICR1* sequence of different *S. cerevisiae* strains in order to understand how the same strain of *S. cerevisiae* could form various types of biofilm. Among the strains analyzed, some were heterozygote at the *FLO11* locus, while others presented two different alleles of *ICR1* (wild type and a 111 bp deletion). We could not find a strong link between strain genotypes and velum characteristics. The same strain in different wines could form a velum having very different characteristics, highlighting a matrix effect.

**II.WS 7****YEAST DIVERSITY IN *VITIS LABRUSCA* L. ECOSYSTEMS****Alberto Luis ROSA**

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**Keywords:** *Vitis*, labrusca, yeast, biodiversity

Although there are detailed studies on the microbiota of *Vitis vinifera* L. grapes, little is known about the diversity of yeast communities present in non-*vinifera* *Vitis* ecosystems (i.e., grapes and spontaneously fermenting grape musts). Potentially scientific and/or enological valuable yeast strains from these non-*vinifera* *Vitis* ecosystems might never be isolated from *V. vinifera* L. Using a standard culture-dependent strategy, we studied the population of yeast species during initial stages of spontaneous fermentation of *V. labrusca* L. (*Isabella*) grape musts. Rare non-*Saccharomyces* yeast species were recognized in *Isabella*, including *Candida azymoides*, *Pichia cecembensis*, *Candida californica*, *Candida bentonensis*, *Issatchenkia hanoiensis* and *Candida apicola*. Interestingly, *P. cecembensis*, not previously recognized in *V. vinifera* grapes or musts, was also found in *V. labrusca* L. grapes in Portugal (Azores Archipelago). Thus, this yeast species could be specifically associated with *V. labrusca* L. grapes, regardless of their geographic origin and/or the associated human interventions. Moreover, *I. hanoiensis*, a yeast species rarely isolated in *V. vinifera* grapes, was also identified in *V. labrusca* ecosystems from Argentina and Portugal. These results suggest that specific *Vitis*-microbial interactions may underlie the assembly of specific grape vine yeast communities. Also interestingly, some yeast genera commonly isolated from *V. vinifera* ecosystems (e.g., *Hanseniaspora*, *Torulaspota* and *Metschnikowia*) were rarely identified and almost never dominated the yeast communities in the *V. labrusca* L. musts we analyzed. Our results reinforce the research interest in biodiversity and extraordinary wine yeasts in ecological niches alternative to traditional *V. vinifera* ecosystems.

**II.WS 8****INFLUENCE OF CELL-CELL CONTACT ON YEAST INTERACTIONS AND EXO-METABOLOME****Clément PETITGONNET**

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**Keywords:** Interactions, Yeast, Flow cytometry, Exo-metabolome

Alcoholic fermentation is the main step for winemaking, mainly performed by the yeast *Saccharomyces cerevisiae*. But other wine yeasts called non-*Saccharomyces* may contribute to alcoholic fermentation and modulate the wine aroma complexity. The recurrent problem with the use of these non-*Saccharomyces* yeasts is their trend to die off prematurely during alcoholic fermentation, leading to a lack of their interesting aromatic properties searched in the desired wine. This phenomenon appears to be mainly due to interactions with *S. cerevisiae*. These interactions are often negatives but remain unclear because of the species and strain specific response. Among the non-*Saccharomyces* yeasts, *Lachancea thermotolerans* is a wine yeast naturally found in grape must and well known as a great L-lactic acid producer and an aromatic molecules enhancer, but its behavior during alcoholic fermentation can be completely different in co-fermentation with *S. cerevisiae* in function of strain used. Thus, *S. cerevisiae*/L. *thermotolerans* couple was used to unravel interactions between these two species during alcoholic fermentation. Thanks to a modified *S. cerevisiae* strain expressing a GFP allowing discrimination between yeast populations, both yeast viability was monitored by flow cytometry in pure and sequential fermentations of grape must with or without cell-cell contact. This reveal a decrease in cell viability for *S. cerevisiae* in both conditions with a greater decrease in case of cell-cell contact. Investigating the nature of the interactions, our results demonstrate a competition between species for nitrogen compounds, oxygen and for the first time a competition for must sterols. Volatile compounds analysis revealed changes in sequential fermentations compared to pure fermentations and showed also that cell-cell contact modify yeast metabolism since the volatile compound profile was significantly different from sequential fermentation without cell-cell contact. Yeast metabolism modifications associated with cell-cell contact were confirmed further by analyzing the exo-metabolome of all fermentations by FT-ICR-MS analysis. These analyses show for the first time a specific metabolite production and quantitative metabolite changes linked to each fermentation condition. This study shows that cell-cell contact not only impact cell viability as already reported but deeply changes the yeast metabolism.

**II.WS 9****NON-SACCHAROMYCES YEAST NITROGEN CONSUMPTION AND METABOLITE PRODUCTION DURING WINE FERMENTATION.**Pauline SEGUINOT<sup>1, 2</sup>Vasileios Englezos<sup>3</sup>, Guillaume Bergler<sup>1, 4</sup>, Anne Julien-Ortiz<sup>2</sup>, Carole Camarasa<sup>1</sup>, Audrey Bloem<sup>1</sup>

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**Keywords:** Non-*Saccharomyces* yeast, nitrogen consumption, metabolite production, wine fermentation

Over the last decade, the use of non-*Saccharomyces* yeasts in the winemaking process has been re-assessed and accepted by winemakers. These yeasts can be used to achieve specific objectives such as lowering the ethanol content, preventing wine spoilage and increasing the production of specific aroma compounds. Since these species are unable to complete alcoholic fermentation, strategies of co- and sequential inoculation of non-*Saccharomyces* and *Saccharomyces cerevisiae* have been developed. However, when mixed starter cultures are used, several parameters (e.g. strain yeast, inoculation timing and nutrient competitions) impact the growth of the individual yeasts, the fermentation kinetics and the metabolites/aroma production. In particular, competition for nitrogen compounds could have a major impact, potentially leading to sluggish fermentation when the yeast assimilable nitrogen (YAN) availability is low. Moreover, many aroma compounds produced by the yeasts are directly produced and influenced by nitrogen metabolism such as higher alcohols, acetate esters and ethyl esters which participate in the organoleptic complexity of wine.

In this context, the first part of this work was to provide an overview of the potentialities of oenological interest of non-*Saccharomyces* species isolated from grape juices. The fermentations were carried out in enological conditions, at 24°C and the potential of several non-*Saccharomyces* yeasts to produce hydrolytic enzymes and metabolites contributing to the sensory properties of wines has been reaffirmed. In particular, the use of *Starmerella bacilliaris* exhibited an increased production of glycerol with a concomitant ethanol decrease. Furthermore, some strains of *Hanseniaspora osmophila* and *Metschnikowia pulcherrima* produced esters and thiols, which may have a positive incidence on the sensory quality of wines.

Then, the nitrogen requirements of non-*Saccharomyces* yeasts were characterized. The analysis of the complete dataset revealed differences between species and even between strains in their preferred nitrogen sources. For example, *S. bacilliaris* strains consumed a limited fraction of amino acids during fermentation while exhausting all the available ammonium. Overall, this work enhanced our understanding of yeasts' nitrogen requirement and metabolism. It also pointed out that an appropriate management of the nitrogen nutrition of yeasts during co- or sequential fermentations to take full advantage of the potentialities of non-*Saccharomyces* species.

**II.WS 10****POPULATION-WIDE DIVERSITY STUDY IN LACHANCEA THERMOTOLERANS HIGHLIGHTS SUPERIOR STARTERS FOR WINEMAKING****Ana HRANILOVIC<sup>1,2</sup>**Vladimir Jiranek <sup>2,3</sup>; Paul R. Grbin <sup>2</sup>; Joanna M. Gambetta <sup>4</sup>; Leigh Schmidtké <sup>4</sup>; Paul K. Boss <sup>5</sup>; Joana Coulon <sup>6</sup>; Isabelle Masneuf-Pomarede <sup>1,7</sup>; Marina Bely <sup>1</sup>; Warren Albertin <sup>1,8</sup>

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**Keywords:** *Lachancea thermotolerans*, non-*Saccharomyces* yeasts, population diversity, wine composition

Grapes from warm(ing) climates often contain excessive sugars but lack acidity. This can lead to highly alcoholic wines with compromised stability and balance. The yeast *Lachancea thermotolerans* can ameliorate such wines due to its metabolic peculiarity – partial fermentation of sugars to lactic acid. This study aimed to elucidate the population-wide diversity in *L. thermotolerans*, whilst selecting superior strains for wine sector. An extensive collection of isolates (~200) sourced from different habitats worldwide was first genotyped on 14 microsatellite loci. This revealed differentiation of *L. thermotolerans* genetic groups based on the isolation substrate and geography. The 94 genotyped strains were then characterised in *Vitis vinifera* cv. Chardonnay fermentations. The comprehensive dataset comprised microbial growth and fermentation kinetics, primary metabolites and 90 volatile compounds. The common traits of *L. thermotolerans* strains were their glucophilic character, relatively extensive fermentation ability (>7.3% v/v EtOH), low production of acetic acid and formation of lactic acid. A seven-fold variation was observed in concentrations of lactate (1.8 – 12 g/L), significantly affecting the wine pH (3.2 – 3.8). Besides the strain-derived variation (significant effect on 80/114 parameters), the metabolic dataset showed separation of pre-determined *L. thermotolerans* genetic groups. The superior *L. thermotolerans* strains were further evaluated in co-inoculations and sequential inoculations with *Saccharomyces cerevisiae*, required for fermentation completion. The chemical and sensory modulations in wines further highlighted the potential of *L. thermotolerans* strains to produce 'fresher' wines with lower ethanol content and improved flavour/balance.

## II.WS 11

# BIOPROTECTIVE NON-SACCHAROMYCES YEAST AS AN ALTERNATIVE TO SULFITES FOR THE WINEMAKING PROCESS

**Sara WINDHOLTZ**

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**Keywords:** sulfites, bioprotection, non-*Saccharomyces*, microbial community

Sulfur dioxide (SO<sub>2</sub>) is used in winemaking due of its antioxidant, antioxydasic and antiseptic properties. Excessive amount of SO<sub>2</sub> can negatively impact wine sensory perception and be detrimental for health. Agri-food industries are more transparent towards consumers concerning addition of sulfites, and oenology is no exception in this clairvoyance. As a consequence, the increase of consumers preference for wine with low or absent of sulfites addition is notorious. In this context, the impact of low/zero sulfites winemaking process on the microbial community should be evaluated. Moreover, microbial agents corresponding to bioprotective cultures represent a growing interest as an alternative to sulfites preservation in the early stages of vinification. However, scientific studies conducted to demonstrate their real effect are almost rare.

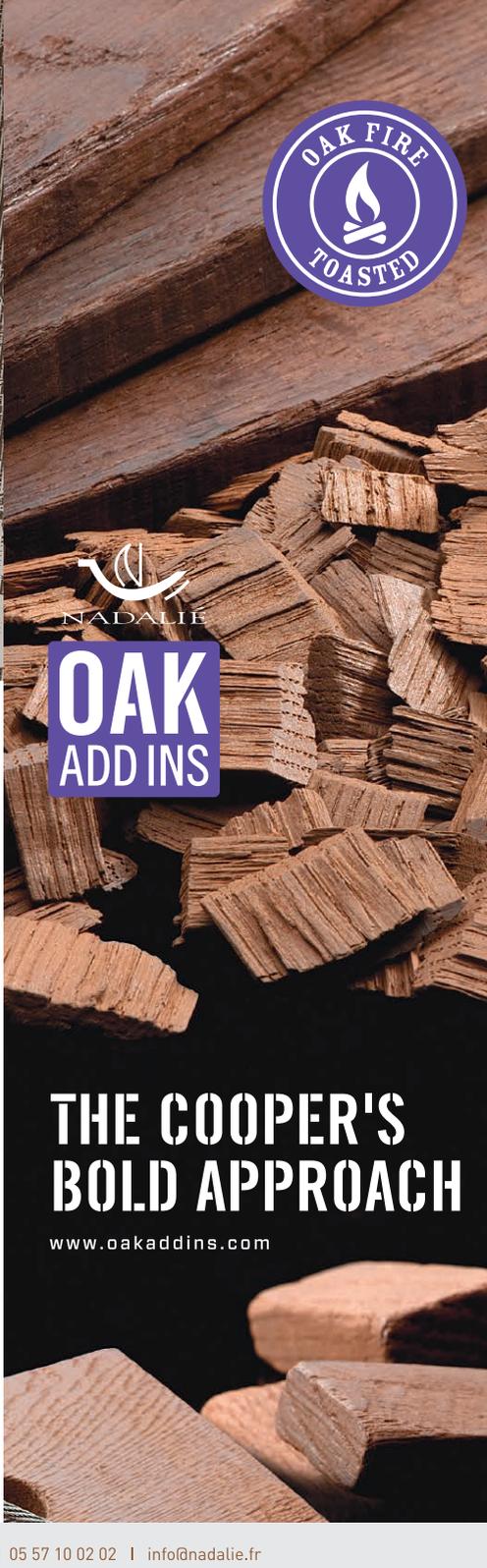
The aim of this study was to evaluate the bioprotection efficiency of non-*Saccharomyces* yeasts as an alternative to the antimicrobial effect of SO<sub>2</sub>. Experiments at different scales (winery, semi-industrial and laboratory) were implemented during two consecutive vintages. Three different treatments: without SO<sub>2</sub>, with SO<sub>2</sub> and bioprotection (mix of *Torulasporea delbrueckii* (Td) and *Metchnikowia pulcherrima* (Mp)) were compared. Population dynamics of targeted microorganisms (Td, Mp, acetic acid and lactic acid bacteria and *Hanseniaspora spp.*) were monitored by qPCR throughout the pre-fermentary stages and the alcoholic fermentation. In a second step, biodiversity of the fungi community was evaluated by high-throughput 18S sequencing using the Illumina MiSeq. qPCR data confirmed that the implantation of the bioprotection non-*Saccharomyces* was effective in all treatments and no significant effect on *Hanseniaspora spp.* population was found. However, a negative effect on the population levels of acetic acid bacteria was showed during the prefermentary stages, higher than sulfiting. Regarding the diversity indices, lower values were obtained for the bioprotection modalities compared with the others, which correlates well with the population dynamics observed previously. Bioprotective cultures seem to represent a promising alternative to SO<sub>2</sub> for niche occupation during the early stage of the winemaking process.





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# Enological Practices and Process Workshop on Wine Stabilization

### III.WS 1

## COLLOIDAL STABILIZATION OF YOUNG RED WINE BY ACACIA SENEGAL GUM: THE MAJOR IMPLICATION OF PROTEIN-RICH ARABINOGALACTAN-PROTEINS

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**Keywords:** Colloidal stabilization, Acacia gum, Coloring matter, Young red wine

Acacia senegal gum (Asen) is an edible dried gummy exudate<sup>1</sup> added in young red wines to ensure their colloidal stability, precluding the precipitation of the coloring matter. Asen macromolecules, belonging to the arabinogalactan-protein (AGP) family<sup>2</sup>, are hyperbranched, charged and amphiphilic heteropolysaccharides composed especially of sugars (92–96%) and a small fraction of proteins (1–3%). Asen is defined as a continuum of macromolecules that could be separated into three fractions by hydrophobic interaction chromatography (HIC)<sup>3-4</sup>. HIC-F1 (85–94% of Asen), HIC-F2 (6–18% of Asen) and HIC-F3 (1–3% of Asen) are named and classified in that order according to their protein content, and then a growing hydrophobicity. The efficiency of Asen towards the coloring matter instability is evaluated according to an “efficacy test” that consists to determine the Asen quantity required to prevent the flocculation by calcium of a colloidal iron hexacyanoferrate solution (International Oenological Codex).

In this study, we investigated the stability mechanism of Asen and its HIC fractions towards the iron hexacyanoferrate – calcium and polyphenols flocculation in hydro-alcoholic solutions and unstable young red wine. The AGPs prevented the colloidal instability of both iron hexacyanoferrate salts and polyphenols in hydro-alcoholic solutions and young red wine with a good correlation between results obtained on both systems. The iron hexacyanoferrate salts was stabilized by electrostatic binding of Asen with calcium, the driver of the flocculation. Experiments performed with HIC fractions showed that the functional property of Asen was only determined by the presence of the AGP rich in proteins (HIC-F2 and HIC-F3 fractions containing 6.3 and 13.8% of proteins, respectively). HIC-F1, the major fraction in weight that contained 0.5% of proteins, was thus devoid of colloidal stability properties. The ability of AGP rich in proteins to colloidal stabilize polyphenols was confirmed in a hydro-alcoholic matrix containing polyphenols and unstable young red wines. Moreover, the richer in proteins is the AGP, the best are their colloidal stabilizing properties. The differences observed in the protective activity between AGPs from the three HIC fractions are related to their protein content but also to their related rate of glycosylation that modulates the protein accessibility to its environment, then their physicochemical properties.

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**III.WS 2****EVALUATING ALTERNATIVES TO COLD STABILIZATION IN WINERIES : THE USE OF CARBOXIMETHYL CELLULOSE, POTASSIUM POLYASPARTATE, ELECTRODIALYSIS AND ION EXCHANGE RESINS - THE RESULTS AFTER ONE YEAR IN THE BOTTLE****Encarna GÓMEZ PLAZA**

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**Keywords:** WINE, STABILIZATION, TARTARIC ACID, ADDITIVES

The tartaric stabilization of wines before bottling to avoid the precipitation of tartaric acid salts is an important and common step during wine production. The presence of precipitated salt crystals in bottle wines is detrimental for their quality and even a legal issue in some countries. Cold stabilization is the most common stabilization treatment. Although it has been shown to be effective, it has some significant disadvantages, mainly regarding losses of color and aromas and its high cost. Therefore, other products and methodologies are being introduced in the wineries for the replacement of this process. Some of these new techniques involve the reduction of the ions causing the insolubilization of tartaric acid while others are based in the formation of protective colloids or the inhibition of the crystallization of salts. In this study, white, rosé and red wines have been treated with carboxymethylcellulose, potassium polyaspartate and an ion exchange resin. The tartaric stability of the wines, together with the oenological, chromatic and sensory characteristics were studied after the wines had been stored during one year in the bottle. The results indicate that the use of carboxymethyl cellulose and potassium polyaspartate maintained the best the sensory and chromatic characteristics and the wine stability of the wines in comparison with an untreated control wine. The potassium polyaspartate treated wine being, in general, the wines preferred in a sensory analysis test.

### III.WS 3

## RESEARCH ON THE ORIGIN AND THE SIDE EFFECTS OF CHITOSAN STABILIZING PROPERTIES IN WINE

### Marguerite DOLS-LAFARGUE

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**Keywords:** chitosan, antiseptic, efficiency, side-effects

Fungal chitosan is a polysaccharide made up of glucosamine and N-acetyl-glucosamine and derived from chitin-glucan of *Aspergillus niger* or *Agaricus bisporus*. Fungal chitosan has been authorized as an antiseptic agent in wine since 2009 (OIV) and in organic wine in 2018. At the maximum dose of 10g/hl, it was shown to eliminate *Brettanomyces bruxellensis*, the main spoilage agent in red wines. Fungal chitosan is highly renewable, biocompatible (ADI equivalent to sucrose) and non-allergenic. However, winemakers often prefer to use sulfites (SO<sub>2</sub>), though sulfites are classified as priority food allergens, than chitosan. Indeed, many conflicting reports exist regarding its efficiency and its side effects towards beneficial wine microorganisms or wine taste. These contradictions could be explained by the heterogeneity of the fungal chitosan lots traded, the diversity of the wines (chemical composition, winemaking process), but also, by the recently highlighted huge genetic diversity prevailing in wine microbial species.

The CHITOWINE project (ANR 17-CE21-0006) is based on the collaboration of three academic partners, a technology transfer unit and an industrial partner. It primarily aims to better define the potential and limitations of fungal chitosan use as an antimicrobial agent in wine. The work will first enable to better define the spectrum of fungal chitosan through the screening of a large microbial collection representative of the inter- and intra-specific diversity of wine ecosystem (more than 200 strains in 17 species of yeasts and bacteria). The chemical characteristics essential to the antiseptic activity of fungal chitosan (degree of acetylation, molecular weight, solubility and charge) and the influence of extrinsic parameters of reaction (pH, temperature, and dose) will be also evaluated. In addition, the physiological effects of chitosan will be sought through biochemical, microscopic and transcriptomic tests, to identify, if possible, the molecular targets of chitosan and to understand the sensitivity differences observed, between inter or intra species and between strains in the same species. Based on these results, improved use recommendation will be proposed and evaluated. Analytical methods to guide chitosan use will be developed and optimized.

**III.WS 4****THE AFFINITY OF WHITE WINE PROTEINS FOR BENTONITE IS DEPENDENT ON WINE COMPOSITION AND IS DIRECTLY RELATED TO THEIR THERMAL STABILITY / SENSITIVITY****Céline PONCET-LEGRAND**<sup>1</sup>Eric Meistermann <sup>2</sup>, Frédéric Charrier <sup>3</sup>, Philippe Cottureau <sup>4</sup>, Patrick Chemardin <sup>1</sup>, Aude Vernhet <sup>1</sup>

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**Keywords:** haze formation, fining, protein adsorption, wine matrix

Bentonite fining is commonly used in oenology to remove all or parts of white wine proteins, which are known to be involved in haze formation. This fining is effective, but has disadvantages: it is not selective, thus molecules responsible for aroma are also removed, it causes substantial volume losses, and finally it generates wastes. Over the last decades, the knowledge of wine proteins has increased: they have been identified, their structures are known, some of them have been crystallized.

However, haze formation is not only a question of protein composition and concentration. It depends on many other factors, such as pH, wine composition (polyphenols, polysaccharides,...). Heat or chemical tests used to adjust the bentonite dose often leads to an overestimation, because they aim at removing all the proteins, even the ones that are stable in the range 60–80°C and are not involved in spontaneous haze.

In this study, we analyzed and quantified the proteins in 7 white wines (3 varieties, 4 areas), treated with four bentonite doses ranging from 5 to 80 g/hL. In parallel, samples of wines were heated during 30 minutes at 40, 60 and 80°C and the residual proteins analyzed.

The wines differed in their protein composition. In each wine, when they were present, the proteins were adsorbed on bentonite in this order: chitinase and  $\beta$ -glucanase, Lipid Transfer Protein (LTP), Thaumatin Like (TL) 22 kDa, TL 19 kDa and Invertase.

The adsorption of a given protein was wine dependent. This could be due to wine pH and ionic strength (different in the studied wines), which changes electrostatic interactions that drive the protein adsorption onto bentonite, but also to other differences in composition (ethanol, polysaccharides, polyphenols, metals...). Experiments performed at pH 2.5 indicated that pH is not the only cause of such different adsorption behaviours: indeed adsorption isotherms were different.

Protein adsorption on bentonite was compared to their thermal sensitivity. It was ranked as previously:  $\beta$ -glucanase - Chitinase > TL22 > TL19 - Invertase > LTP. It is worth noting that the most thermostable proteins are the ones which need the highest doses of bentonite on a wide panel of wines. These stable proteins do not need to be removed and thus bentonite doses could be reduced. More specific tests, which would take into account only the most sensitive proteins need to be developed.

### III.WS 5

## IMPORTANCE OF MATRIX EFFECTS (WINE COMPOSITION) ON PROTEIN STABILITY TESTS OF WHITE AND ROSÉ WINES

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The presence of unstable proteins in wines can affect their stability and clarity. Before bottling, winemakers need to be sure that the wine is stable. A large number of stability tests have been proposed, usually based on heating a sample with a specific time-temperature couple. In practice, none is effective to accurately assess the risk of instability. Moreover, the interpretation of the results of these tests changes according to the region.

The aim of this work is to compare, on 55 wines (4 vintages, 7 varieties, 5 areas), the most common heat test (30 minutes at 80°C) with the turbidity measured after 15 days at 35°C on bottled wines. Proteins were analyzed in 33 cases. In addition, 10 wines were heated at 40°C/30 min, 40°C/4 hours, 35°C/15 days and 80°C/30 min and the residual proteins analyzed.

The results show no correlation between turbidity after heat test 80°C/30 min and after 15 days at 35°C. For some wines, especially Gewurztraminer ones, turbidity after heating at 80°C can reach 330 NTU without any visual haze at 35°C (< 3 NTU). Similar results are obtained when the heat test is performed after adjustment of pH to 3.4. The turbidity after heat test 80°C/30 min increases with pH, particularly above 3.6, which is not so unusual for Gewurztraminer wines. The pH effect is less significant at 40°C. Finally, pH values alone cannot explain the different behaviors of wines.

On the other hand, protein composition in wines depends on their pH. Thaumatin Like proteins (TL) 19 kDa, TL 22kDa and Invertases are present in almost all wines. Half of them contains Lipid Transfer Protein (LTP) and only a few Chitinases and  $\beta$ -Glucanase. These proteins are present when pH is lower than 3.5, probably because low pH favor Chitinase and  $\beta$ -glucanase conformational changes and precipitation.

Protein analysis after heating these various wines at different time-temperature couples led to this ranking:

Chitinases are sensitive at low temperature (40°C) and resist better at pH 3.7;

TL 22kDa are sensitive, especially in Rosé wines;

TL 19kDa are more stable, but their sensitivity depends on the pH;

Invertase unfold between 60 and 80°C but is not affected by the pH;

LTP can resist up to 80°C.

Turbidity after usual heat test 80°C/30 min increases with total proteins concentration and pH. This is not observed after 15 days at 35°C or 4 hours at 40°C. These tests may be better to evaluate the actual risk of instability after bottling.

**III.WS 6****REMOVAL OF WHITE WINE HEAT UNSTABLE PROTEINS BY USING PROTEASES AND FLASH PASTEURIZATION-COMPARISON WITH BENTONITES TREATMENTS****Richard MARCHAL**

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White wine protein haze can be prevented by removing the grape juice proteins, currently achieved by bentonite addition. To avoid wine volume loss and to minimize aroma stripping, degrading haze-forming proteins in wine with proteases is a particularly interesting alternative to bentonite.

In the present study, two fungal proteases treatments combined with different heating (50, 60, 72°C) + refreshing steps, were applied on Gewürztraminer grape juice, and compared to bentonite treatments. The impact of these 19 treatments on the wine haze risks was determined by using two heat tests at 50°C (heating during 30 to 120 min) and 80°C (heating during 5 to 60 min). The protein contents and compositions were also estimated using the SDS-PAGE + densitometric integration techniques.

The heat instability tests of the 19 wines show strongly different results according to the test used. With the 50°C heating tests, the wines showed logarithmic curves with a maximal value reached in 30 min. At the opposite, after the 80°C heating tests, the white wines showed a linear increase of the turbidity during the 60 min of the heating, leading to linear curves with  $R^2 > 0.99$ . Moreover, the turbidities observed were much higher when the wines were heated at 80°C when compared with the wines after the 50°C tests. These results clearly pointed out the discrepancies between the test selected to estimate a white wine haze risk and the treatment necessary to avoid a haze after bottling.

Concerning the wines obtained after juice bentonite treatments, we observed a dose effect with a high correlation at 50°C between the dose of swelling clay and the wine haze risk. 60 g/hL were necessary to reach the colloidal stability, whatever the test used (50 or 80°C) and the heating time. The addition of proteases at 50°C or 60°C during 1 hr before a quick increase at 72°C (as recommended by the OIV) and refreshing in cold water decreased the haze risk by 75% and 85% respectively when compared to the control wine, whilst the same heat treatment without enzymes only decreased the haze risk by 28% and 17% respectively.

The ability for enological proteases to hydrolyze grape berry heat unstable proteins (observed by SDS-PAGE) was strongly evidenced with the heat test at 50°C. Proteases reduced the heat instability by 40% whilst the heat treatment alone was pretty ineffective.

This study proved the possibility to use proteases as an efficient treatment to control white wine haze risk.

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# Analysis and composition of grapes, wines, wine spirits

#### IV.SC 1

## CORRELATION BETWEEN SKIN CELL WALL COMPOSITION AND PHENOLIC EXTRACTABILITY IN CABERNET SAUVIGNON WINES

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**Keywords:** Extractability, Cell wall, Phenolics, Red wine

The phenolic component of red wine is responsible for important elements of flavor and mouthfeel, and thus quality of the finished wine. Additionally, many of these phenolics have been associated with health benefits such as reduction of the risk of developing cardiovascular disease, cancer, osteoporosis and preventing Alzheimer's disease. While the origins, concentrations, and chemistries of the phenolics in a finished red wine are well known, the fundamental mechanisms and kinetics of extraction of these phenolics from grape skins and seeds during red wine fermentation are poorly understood. This lack of knowledge regarding the extraction mechanisms of phenolics during red wine fermentation makes informed manipulations of the finished wine's phenolic composition difficult.

The skin cell walls of berries play a very important role during the winemaking process as they can form a barrier to release of important flavor compounds, and is a potential adsorption surface. Commercial wineries have observed that polyphenol extraction levels during winemaking may vary based on grape growing region and/or site. Cell wall composition may be one of the important factors influencing this relationship.

In this work, phenolic extractability of Cabernet Sauvignon from two regions within California (Sonoma and Central Coast) has been studied. The study includes the analysis of phenolic berry composition, wine phenolic content as well as skin cell wall composition of three sites per region. Results showed that berry phenolic content is not directly related to the region where the grapes were grown. Within the same region, sites with high and low phenolic berry amounts were found. Regarding the wines, a relationship between region and phenolic content was found. Wines made from Central Coast grapes presented lower phenolic content than those from Sonoma. In order to understand the connection between wine phenolic content and extractability, skin cell wall material was characterized. Partial least squares (PLS) analysis showed that cellulose and uronic acid content might influence the extractability of phenolics during fermentation.

## IV. SC 2

## DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR THE QUANTIFICATION OF TERPENS IN WINES

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**Keywords:** DLLME, Terpens, Alcoholic fermentation, Wine yeast

In a highly competitive worldwide market, a current challenge for the beverage sector is to diversify the range of products and to offer wines and spirits with typicity and character.

During alcoholic fermentation, wine yeasts generate a large variety of volatile metabolites, including acetate esters, ethyl fatty acid esters, higher alcohols, volatile fatty acids and volatile sulfur compounds that contribute to the aroma profile of wine. These molecules, referred as fermentative aromas, are the most abundant volatile compounds synthesized by yeasts and the metabolic pathways involved in their formation have been well characterized. Furthermore, other molecules with a major organoleptic impact may be produced during wine fermentation including terpene derivatives. However, little information is available on the contribution of yeasts to the formation of these molecules, in particular on their ability to synthesise *de novo* the terpens derivatives or to produce hydrolytic enzymes involved in the release of varietal precursors.

To study the yeasts ability to produce these molecules, a dispersive liquid-liquid microextraction (DLLME) gas chromatography mass spectrometry was developed for their quantification in white wines, synthetic wine and fermented synthetic medium. A mixture of acetone (dispersive solvent) and dichloromethane (extractive solvent) was added to 5 ml of sample. The proposed method showed no matrix effect, a good linearity in enological range (from 10 to 300 µg/L), good recoveries, inter-day precision and good reproducibility. The developed method was applied to the analysis of the capacities of 41 yeast strains to produce terpene compounds in Chardonnay must and in synthetic medium. Interestingly, the majority of the studied compound has been detected and quantified in the resulting wines.

This sample-preparation technique is very interesting for high-throughput studies and for economic and environmental reasons because it is fast, easy to operate with a high enrichment, and consumes low volume of organic solvent.

#### IV. SC 3

### IMPACT OF PRESS FRACTIONING ON PINOT NOIR AND PINOT MEUNIER GRAPE JUICE AND WINE COMPOSITIONS AND COLOUR.

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**Keywords:** Press fractioning, grape juice, sparkling base wine colour, ACP

The separation of different grape juice press fractions is an important step in the production of sparkling base wines. A complete press cycle for this style of wine is a series of pressure increases (squeezes) resulting in variations in juice composition during the press cycle. After alcoholic fermentation, wines obtained from grape juices also exhibit strong differences for numerous characteristics. Nevertheless, there is no statistical study of the impact of the press cycle on grape juices and wine colour/composition. So, the aim of this study (vintage 2018) was to investigate the changes in composition and colour parameters of Pinot noir and Pinot meunier grapes juices, as well as their corresponding wines, during the pressing cycle.

The studied parameters were:  $L^*a^*b^*$ ,  $A_{420}$ , pH, total acidity (TA), malic and tartaric acids, sugars, maturity index, YAN,  $NH_4^+$ ,  $a-NH_2$ ,  $Ca^{++}$  and  $K^+$  for the 23 grape juices, and pH, TA, malic and tartaric acids, alcohol,  $a-NH_2$ ,  $Ca^{++}$  and  $K^+$  for the 23 base wines. Results were analysed by Pearson's correlation test, ANOVA and PCA after normalization of the data.

For examples, the TA and the tartaric acid content of the musts statistically decreased by 35% and 41% respectively between the beginning and the end of the press cycle, whilst the pH increased by 0.4 unit. These changes were observed concomitantly with the increases of  $a^*$  and  $b^*$  values by 4 to 6 units and a significant decrease of the luminosity  $L^*$ . These observations were still true for wines. Many Pearson's correlation coefficients were higher than 0.85 and even higher than 0.95 for some of them. The different PCAs considering the colour parameters, the acidity parameters or all of the parameters measured showed a strong separation of the samples according to the different squeezes, for grape juices as well as for the wines. This was confirmed with the PCA considering the 23 grape juices, the 23 wines and all of the parameters measured both in juice and wines.

As a conclusion, this study brings a greater understanding of: 1) Pinot noir and Pinot meunier must composition and colour changes all along the press cycle, 2) differences between wines produced with these grape juice fractions, 3) correlation between grape juice and wine compositions. These results could be a good tool for winemakers to decide how to separate the grape juice fractions during the pressing cycle to produce different styles of wines with different sensory qualities and aging potential.

## IV. SC 4

## IDENTIFICATION OF CIS-2-METHYL-4-PROPYL-1,3-OXATHIANE AS A NEW VOLATILE SULFUR COMPOUND (VSC) IN WINE

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**Keywords:** wine aroma, tropical fruit, 3-sulfanylhexan-1-ol, stable isotope dilution assay

Despite their trace concentrations, volatile sulfur compounds (VSCs) are an important category of flavour-active compounds that significantly contribute to desirable or undesirable aromas of many foods and beverages. In wines, VSCs in the form of polyfunctional thiols, notably 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA), and 4-sulfanyl-4-methyl-pentan-2-one (4-MSP), possess extremely low olfactory thresholds ( $\approx$  ng/L) and pleasant "tropical aroma" notes. They have received much attention with respect to their sensory contributions, quantitative occurrences, biogenesis, and thiol management through viticulture and winemaking. However, the fate of these potent volatiles are still not fully understood.

In this work [1], the fate of 3-SH in wine was probed based on the potential sensory and chemistry interactions between 3-SH and acetaldehyde, which led to the identification of cis-2-methyl-4-propyl-1,3-oxathiane (cis-1) in wine for the first time. Subsequently, a stable isotope dilution assay (SIDA) using headspace-solid-phase microextraction with gas chromatography and mass spectrometry (HS-SPME GC-MS) was developed. A range of parameters was optimised, a deuterated internal standard was synthesised, and the method was fully validated and applied to the quantitation of cis-1 in wines. The aroma detection threshold of 1 was also determined.

The existence of cis-1 in Sauvignon blanc wines from a laboratory-scale fermentation trial was revealed by mass spectral comparison to an authentic standard, linear retention indices of naturally present cis-1 on two GC columns, and co-injection experiments. Challenges were faced when analysing commercial wine samples due to unknown co-eluting interferences in some wines. After employing d4-1 as the internal standard and evaluating additional capillary column phases, a sensitive SIDA HS-SPME GC-MS method was developed and applied to a survey of commercial wines. Interestingly, trans-1 was not detected whereas cis-1 ranged from undetectable to 460 ng/L, which highly correlated ( $r = 0.72$ ) to the concentrations of 3-SH, determined in the same wines by HPLC-MS/MS after derivatisation. The aroma detection threshold of 1\* in a neutral Australian white wine was found to be 7.1  $\mu$ g/L. Although cis-1 concentrations in the studied wines were below the odour detection threshold of 1, our results suggest a potential link from cis-1 to the potent VSC 3-SH, and more research is required to gain a better understanding of the importance of cis-1 in wine, from both chemistry and sensory perspectives. The identification and method development work will be presented along with additional experiments involving cis-1 in wine.

[1] Chen, L., D.L. Capone, and D.W. Jeffery, Identification and Quantitative Analysis of 2-Methyl-4-propyl-1,3-oxathiane in Wine. *Journal of Agricultural and Food Chemistry*, 2018, 66 (41), 10808–10815.

\* Using a commercial standard consisting of 85% cis-1 and 15% trans-1.

#### IV. SC 5

### COMPARISON OF TANNIN ANALYSIS BY PROTEIN PRECIPITATION AND NORMAL-PHASE HPLC

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**Keywords:** Tannin analysis, Protein Precipitation Assay, NP-HPLC

Tannins are a heterogeneous class of polymeric phenolics found in grapes, oak barrels and wine. In red wine tannins are primarily responsible for astringency, though they also have an important role in reacting with and stabilizing pigments. There are numerous sub-classes of tannins found in wine but they all share structural heterogeneity within each sub-class, with varied polymer composition, configuration and length. Numerous methodologies exist for the quantification of tannins, however, protein precipitation using bovine serum albumin has proved itself useful due to its strong correlation to the sensory perception of astringency and the basic instruments required for the method. Though the method can yield valuable insights into tannin composition, it cannot be automated easily and necessitates well-trained personnel. RP-HPLC analysis has been used for the quantification of low molecular phenolic compounds for a long time, but it is not suitable for the quantification of tannins. A normal-phase (NP)-HPLC method using a ternary solvent system is suggested, which is able to separate the phenolic compounds from red wine into three major fractions. Comparison with standard phenolic compounds allowed the characterization and quantification of these fractions and the results were compared to those obtained by protein precipitation.

**Workshop**  
**Analytical tools using**  
**electromagnetic**  
**spectroscopy techniques (IR,**  
**fluorescence, Raman)**

**IV.WS 1****DETERMINATION OF TITRATABLE ACIDITY, SUGAR AND ORGANIC ACID CONTENT IN RED AND WHITE WINE GRAPE CULTIVARS DURING RIPENING BY VIS-NIR HYPERSPECTRAL IMAGING****Mario Gabrielli - CHANTAL MAURY**

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**Keywords:** grape, Hyperspectral imaging, ripeness, non-destructive analysis

Grape harvest time is one of the most fundamental aspects that affect grape quality and thus wine quality. Many factors influence the decision of harvest; among them technological and phenolic maturity of grape. Technological ripeness is mainly related to sugar concentration, titratable acidity and pH. Conventional methods for chemical analysis of grapes are normally sample-destructive, time-consuming, include laborious sample preparation steps, and generate chemical waste, thereby limiting their utility in online/in-line quality monitoring. Moreover, destructive analyses can be performed only on a limited number of fruit pieces and, thus, their statistical relevance could be limited. This study evaluated the ability of a lab-scale hyperspectral imaging (HYP-IM) technique to predict titratable acidity, organic acid and sugar content of grapes. Samples of Cabernet franc and Chenin blanc grapes were consecutively collected six times at weekly intervals after veraison. The images were recorded thanks to the hyperspectral imaging camera Pica L (Resonon) in a spectral range from 400 to 1000 nm. Statistics were performed using Microsoft XLstat software. Successively, the berries were analyzed for their sugar (glucose and fructose) and organic acid (malic and tartaric acid) content and titratable acidity according to usual methods.

The raw spectra recorded were pre-treated with the following external procedures: Standard Normal Variate (SNV); 1st Derivative (1stDER); 2st Derivative (2stDER); and White and Black (W-B) correction. A quantitative model was developed using partial least squares regression (PLS-R) in order to find correlations between spectra information and each of the chemical references. Preliminary results showed a good correlation between each of the chemical parameters and the spectral information. The best model was obtained using 1st DER data pre-treatment, yielding the validations coefficients (P-R<sup>2</sup>) of 0.972, 0.932, 0.921 and a root mean square error of prediction (RMSEP) of 0.249, 3.619, 0.140 for titratable acidity, sugar and organic acid content, respectively. Therefore, hyperspectral systems can be a fast and non-destructive promising technology to predict the levels of titratable acidity, sugar and organic acid content in wine grapes during the ripening and at harvest time.

**VI.WS 2****USE OF FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) TO RAPIDLY VERIFY THE BOTANICAL AUTHENTICITY OF GUM ARABIC****Mario MALACARNE**

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**Keywords:** gum arabic, FT-IR, botanical origin, authenticity

Gum arabic is composed of a polysaccharide rich in galactose and arabinose along with a small protein fraction [1, 2], which gives its stabilizing power with respect to the coloring substances or tartaric precipitation of bottled wine. It is a gummy exudation from Acacia trees; the products used in enology have two possible botanical origins, i.e. Acacia seyal and Acacia senegal, with different chemical-physical features and consequently different technological effects on wines. The aim of this work is to evaluate the feasibility of discrimination of commercial gums Arabic between their two different sources, on the basis of the absorption of the Fourier Transform Infrared (FT-IR) spectra of their aqueous solutions, in order to propose an extremely rapid and cost-saving method for quality control laboratories.

Forty five samples of commercial gum Arabic were collected on the Italian market of enological products and their botanical origin (Acacia seyal, N=30; Acacia senegal, N=15) were established by applying the reference method recommended by the International Organisation of Vine and Wine [1], based on the total nitrogen content and the rotatory power. After a dilution to obtain 5% of dry matter aqueous solutions, FT-IR spectra of samples were acquired in the 926–5011  $\text{cm}^{-1}$  range with a resolution of 3.8  $\text{cm}^{-1}$ , and a statistical approach was applied on the FT-IR spectra to verify the ability to distinguish gums Arabic from the two botanical origins. Standard Discriminant Analysis correctly classified all the samples, providing an optimal distinction between the 2 botanical origins on root 1. The robustness of the model was verified using an external validation. For this aim the entire dataset was divided into a 'training' dataset, 80% of samples for the 2 categories, and a 'validation' dataset, the remaining 20%. The model was built using the training dataset and then the validation samples were classified on it and this process was repeated 3 times. In all cases, 100% of correct classification was obtained.

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## VI.WS 3

**MONITORING SMALL-SCALE ALCOHOLIC FERMENTATIONS USING A PORTABLE FTIR-ATR SPECTROMETER AND MULTIVARIATE ANALYSIS****Julieta CAVAGLIA**

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**Keywords:** Process Control, Alcoholic Fermentation, FTIR, Portable device

Although some wine production processes still rely on post-production evaluation and off-site laboratory analysis, the new winemaking industry is aware of a need for a better knowledge of the process to improve the properties of the final product. Thus, more and more wineries are interested in incorporating quality-by-design (QbD) strategies instead of postproduction testing because of the possibility to early detect deviations in fermentation or any other wine process. This would allow to detect unwanted situations and eventually to 'readjust' the process, thus minimizing rejects.

A strategy consisting on coupling FTIR-ATR spectroscopy and multivariate analysis is here proposed as a fermentation process control strategy. The idea was to develop a portable, rapid, easy-to-use and economic device/tool to monitor fermentation processes and to detect deviations from the normal fermentation conditions (NFC). A portable FTIR-ATR spectrometer was used to monitor small-scale alcoholic fermentations (microvinifications), some of them conducted in NFC and some others intentionally deviated from it. FTIR-ATR measurements were collected during the fermentation process and relative density and content of sugars (glucose and fructose), acetic acid, malic acid and lactic acid were analyzed by traditional methods.

Multivariate analysis (exploratory methods and linear regression methods) was applied in order to model the whole fermentation process and detect deviations. The prediction of the sugar content in fermenting samples was achieved, demonstrating the possibility to use this portable device to rapidly monitor fermentations and to detect at an early stage slower fermentations, giving the possibility to the winemaker to eventually correct the process and to obtain a good quality product. Moreover, control charts based on multivariate Hotelling  $T^2$  and  $Q$  statistics were built to detect abnormal deviations. In conclusion, this methodology shows great potential as a fast and simple at-line analysis tool for early detection of fermentation problems.

*Acknowledgment:* The financial support by the Spanish Ministry of Science and Technology (Project AGL2015-70106-R) is acknowledged.

**IV.WS 4****DEVELOPMENT OF FTIR PARTIAL LEAST SQUARES MODELS FOR POLYPHENOL QUANTIFICATION IN RED WINE DURING FERMENTATION****Clément MIRAMONT**

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**Keywords:** Polyphenol, Fourier Transform Infrared, Partial Least Squares regression, Spectroscopy

Polyphenolic compounds are considered to have a major impact on the quality of red wines. Sensory impact, such as astringency and bitterness, stems directly from tannin composition. Thenceforth, quick analytical measurement of phenolic compounds appears to be a real challenge for winemaking monitoring and process control.

Many methods were developed to analyze polyphenols in wine, but they are time-consuming and require chemistry skills and equipment, not suitable for a rapid routine analysis. A reliable and rapid method to obtain this kind of measurement is Fourier Transform Infrared (FTIR) spectroscopy.

Thus, in order to develop new methods based on FTIR spectroscopy, this work first sought to follow polyphenols during winemaking in a vineyard of Bordeaux area, through two different vintages, different type of winemaking and grape varieties. For this purpose, tannin concentration was analyzed by precipitation with Bovine Serum Albumin assay and Methylcellulose assay. In order to obtain the most complete information, the samples were also analyzed by HPLC, using the phloroglucinolysis reaction to obtain the mean degree of polymerization and indication on galloylation, procyanidin and prodelphinidin ratio.

The data collected were statistically analyzed by Partial Least Squares regression method for quantification of laboratory-determined polyphenols from FTIR spectra. Cross validation was used to validate the predictive performance of the models.

Correlations obtained show good results for all parameters studied, with coefficient of determination ( $r^2$ ) for both calibration and cross validation larger than 0.8. This work is the first step for the construction of robust models to quantify different polyphenols parameters during winemaking by FTIR spectroscopy.

## MULTIVARIATE CHARACTERIZATION OF ITALIAN MONOVARIETAL RED WINES USING FTIR SPECTROSCOPY

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**Keywords:** authenticity, FTIR, tannins, red wine

The assessment of wine authenticity is of great importance for consumers, producers and regulatory agencies to guarantee the geographical origin of wines and grape variety as well. Since mid-infrared (MIR) spectroscopy with chemometrics represent a suitable tool to ascertain the wine composition, including features associated with the polyphenolic compounds, the aim of this study was to generate MIR spectra of red wines to be exploited for classification of red wines based on the relationship between grape variety and wine composition. Several multivariate data analyses were used, including Principal Component Analysis (PCA), Discriminant Analysis (DA), Support Vector Machine (SVM), and Soft Intelligent Modelling of Class Analogy (SIMCA).

The aim of this study was to investigate the application of MIR spectroscopy (from 4000 to 700  $\text{cm}^{-1}$ ) combined with multivariate analysis to provide a rapid screening tool for discriminating among different red monovarietal Italian wine varieties.

A total of 110 monovarietal red wines vintage 2016 were collected directly from the companies across different regions of Italy, including the following eleven grape varieties: Sangiovese, Nebbiolo, Aglianico, Nerello Mascalese, Primitivo, Raboso, Cannonau, Teroldego, Sagrantino, Montepulciano and Corvina.

PCA showed five wavelengths that mainly contributed to the PC1, including much-closed peak at 1043  $\text{cm}^{-1}$  that correspond to the C–O stretch absorption bands that are important regions for glycerol, whereas the ethanol peaks at about 1085  $\text{cm}^{-1}$ . The band at 877  $\text{cm}^{-1}$  would be related to C–C stretching vibration of organic molecules, whereas the asymmetric stretching for C–O in aromatic –OH group of polyphenols within the spectral regions from 1050 to 1165  $\text{cm}^{-1}$ . In particular, the (1175) – 1100 – 1060  $\text{cm}^{-1}$  vibrational bands are combination bands involving C–O stretching and O–H deformation of phenolic rings. The 1166–1168  $\text{cm}^{-1}$  peaks are attributable to in-plane bending deformations of C–H and C–O groups of polyphenols, respectively, which polymerization may cause a slight peak shift due to the formation of H-bridges.

The best results were obtained with the SVM that achieved an overall correct classification up to 72.2% for test set, and 44.4% for the validation set of wines, respectively. The Sangiovese wines (n=19) were splitted in two sub-groups (Sang-Romagna n=12; Sang-Tuscany n=7) considering the indeterminacy of its origins, disputed between Romagna and Tuscany. Although the classification of three grape varieties was problematic (i.e. Nerello Mascalese, Raboso and Primitivo), the remaining wines were almost correctly assigned to their actual classes.

In conclusion, MIR spectroscopy coupled with chemometrics represents an interesting approach for the classification of monovarietal red wines, which is important in quality control and authenticity monitoring.

*Acknowledgements: MIUR project N. 20157RN44Y. D-WINE research group: A. Gambuti<sup>(UniNA)</sup>, V. Gerbi<sup>(UniTO)</sup>, F. Mattivi<sup>(FMach)</sup>, L. Rolle<sup>(UniTO)</sup>, M. Marangon<sup>(UniPD)</sup>, D. Perenzoni<sup>(FMach)</sup>, P. Piombino<sup>(UniNA)</sup>, A. Rinaldi<sup>(UniNA)</sup>, B. Simonato<sup>(UniVR)</sup>, G. Tornielli<sup>(UniVR)</sup>, S. Vincenzi<sup>(UniPD)</sup>*

## IV.WS 6

**CIEDE2000 COLOUR DIFFERENCE VALUE AS A PARAMETER FOR TRACING THE AGEING PROCESS ON WOOD AGED SPIRITS****Manuel Jesús DELGADO GONZÁLEZ**

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**Keywords:** Colour, Spirit, Wood, Ageing

It is quite common nowadays to carry out analyses which allow to control the ageing of spirits that are aged in wood casks. Many control parameters have been previously studied, such as the concentration of different phenolic compounds or the Total Polyphenol Index, in order to better understand the ageing process of wood aged spirits. On the other hand, it is frequent to analyse as a physical parameter the colour of those spirit samples, by stating them as an array of three coordinates from various colour spaces as CIE L\*a\*b\* or CIE L\*C\*H\*.

In year 2001, the International Commission of Illumination proposed and/or modified various mathematical formulas for measuring the colour difference between two different samples and named that parameter as CIEDE2000. This value allows to quantify, with a number within a range from 0 to 100, the visual difference between two colours and, at the same time, it establishes some value ranges which give some information about how easy is by an observer to differentiate them by eye.

Due to the ageing process in wood casks of alcoholic beverages produces changes on the intensity and on the hue of the colour, in the present work we proposed to study, by analysing the colour differences between various samples aged in different times, if the CIEDE2000 parameter could be used as a parameter on the tracing of the ageing process.

To this end, kinetical analyses and statistical regressions were carried out over different wood-aged spirits samples, obtaining good R<sup>2</sup> values in return, stating that colour difference values could be used as parameters to study and better comprehend the ageing process of beverages in wood casks.

**IV.WS 7****MONITORING GAS-PHASE CO<sub>2</sub> IN THE HEADSPACE OF CHAMPAGNE GLASSES THROUGH DIODE LASER SPECTROMETRY****Anne-Laure MORIAUX (1)**

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**Keywords:** Champagne, CO<sub>2</sub>, Diode laser spectrometry, Tasting conditions

During Champagne or sparkling wine tasting, gas-phase CO<sub>2</sub> and volatile organic compounds invade the headspace above glasses [1], thus progressively modifying the chemical space perceived by the consumer. Gas-phase CO<sub>2</sub> in excess can even cause a very unpleasant tingling sensation perturbing both ortho- and retronasal olfactory perception [2]. Monitoring as accurately as possible the level of gas-phase CO<sub>2</sub> above glasses is therefore a challenge of importance aimed at better understanding the close relationship between the release of CO<sub>2</sub> and a collection of various tasting parameters.

Based on the tunable diode laser absorption spectroscopy (TDLAS), a diode laser spectrometer (namely, the CO<sub>2</sub>-DLS) dedicated to monitor gas-phase CO<sub>2</sub> in the headspace of champagne glasses was developed [3,4]. The concentration of gas-phase CO<sub>2</sub> found in the headspace of champagne glasses served under multivariate conditions was accurately monitored, all along the first 10 minutes following the action of pouring. Our results show the strong impact of various tasting conditions (such as the volume of wine dispensed, the glass shape, the wine temperature, or the level of effervescence, for example) on the release of gas-phase CO<sub>2</sub> above the champagne surface. Moreover, a recent upgrading of the CO<sub>2</sub>-DLS allowed us to evidence that the concentration of gas-phase CO<sub>2</sub> in the headspace of a champagne glass is far from being homogeneous in either space or time, with much higher gas-phase CO<sub>2</sub> concentrations close to the wine interface.

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**VI.WS 8****DISCRIMINATION OF WHITE WINES BY RAMAN SPECTROSCOPY COUPLED WITH CHEMOMETRIC METHODS****Chantal MAURY**

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**Keywords:** white wines, authenticity, Raman spectroscopy, chemometrics

France is the largest exporter of wine in the world. The export turnover is estimated at 8.7 billion euros in 2017 for 13 million hectoliters sold. This lucrative business pushes scammers to increase the value of some low-end wines by cheating on their appellations, quality or even their origins. These facts lead to losing 1.3 billion euros each year to the European Union's wine and spirits companies.

The control of wine quality is performed by analytical methods such as infrared, NMR or HPLC. Nevertheless, the presence of water and ethanol interferes with the determination of the other wine molecules. In addition, the complexity of the wine matrix and the chemical similarity between its main compounds complicate the extraction of information obtained by these analytical methods. Consequently, the need to develop more sensitive, fast and automated procedures remains a real need for investors and stakeholders in this area. Our study aims to evaluate the ability of Raman spectroscopy to discriminate wines depending on their origin and grape variety based on their spectral fingerprints. Wines from 8 grapes varieties have been studied: Chardonnay (Bourgogne), Riesling (Alsace), Gewurztraminer (Romania), Muscadet (Val de Loire), Sauvignon blanc (Bordeaux), Muscat (Pays d'Oc) and a blend with Semillon (Bergerac). The results showed that white wine has a rich spectral signature (excitation at 532 nm) which reflected its molecular composition. The application of statistical tests (Kruskal-Wallis) made it possible to classify 6 different groups thus confirming that the spectra of the analyzed wines are different. Principal component analysis and discriminant analysis showed a perfect discrimination between the different wines. The validation of the database with another wine that is not part of the model (Sauvignon blanc, Val de Loire) showed a very good discrimination between the different wines. Nevertheless, confusion was observed between the two Sauvignon because the model could not differentiate them despite their different origins.

Raman spectroscopy allows the rapid identification of the grape variety. Nevertheless, a large number of samples must be analyzed in order to evaluate the industrial viability of this technique (variability between years, batches) and validate the approach on a large panel of wine belonging to grape varieties and different geographical areas.



# **Chemical and Biochemical reactions, including grape and wines microorganisms impact**

## V.SC 1

# FRUCTOSE IMPLICATION IN THE SOTOLON FORMATION IN FORTIFIED WINES: PRELIMINARY RESULTS

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**Keywords:** sweet wines, thermal processing, wine ageing, wine models

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) is a naturally occurring odorant compound with a strong caramel/spice-like scent, present in many foodstuffs. Its positive contribution for the aroma of different fortified wines such as Madeira, Port and Sherry is recognized. In contrast, it is also known to be responsible for the off-flavor character of prematurely aged dry white wines. The formation mechanisms of sotolon in wine are still not well elucidated, particularly in Madeira wines, which are submitted to thermal processing during its traditional ageing. The sotolon formation in these wines has been related to sugar degradation mechanisms, particularly from fructose [1].

The present study focuses on the LC-MS/MS quantification results of sotolon [2] in model wines and serves as preliminary results in a currently ongoing project, with the aim to elucidate the main formation pathways of sotolon in fortified wines. Different combinations of fructose and amino acids in synthetic wine (base wine model - 6 g/L of tartaric acid, 18% ethanol (v/v) and pH adjusted to 3.5) were tested and submitted to forced ageing at 70 °C for 1 month. The results showed that fructose levels as low as 1 g/L are enough to produce sotolon levels higher than the odour detection threshold preliminary found for Madeira wines [3]. Results also showed that cysteine somehow hindered the sotolon formation in model wines.

## ACKNOWLEDGMENTS

Vanda Pereira is thankful to ARDITI (Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação) for funding her research grant through the project M1420-09-5369-FSE-000001. This work was financed by ERDF funds and National Funds through the FCT (Fundação para a Ciência e Tecnologia) under the projects UID/CTM/50025/2013 and M1420-01-0247-FEDER-000020 (IMPACT III).

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## V.SC 2

**VARIETAL DIFFERENCES BETWEEN SHIRAZ AND CABERNET SAUVIGNON WINES REVEALED BY YEAST METABOLISM****Guillaume ANTALICK**

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**Keywords:** wine volatiles, berry maturity, yeast, metabolism

This study investigated if compositional differences between Shiraz and Cabernet Sauvignon grape varieties could influence the production of yeast-derived compounds. This work was based on the analysis of 40 experimental red wines made in triplicate fermentations from grapes harvested from two consecutive vintages in New South Wales (Australia). Grapes were picked at three maturity stages using berry sugar accumulation as physiological indicator, from nine commercial vineyards located in three different climatic regions (temperate, temperate-warm and warm-hot). A range of 30 yeast-derived wine volatiles including esters and alcohols were quantified by HS/SPME-GC/MS. Ammonia, amino-acids and lipids were analysed in the corresponding grapes. The juice total soluble solids (°Brix) in addition to the wine alcohol and residual sugar levels were also measured. The influence of grape maturity on wine ester composition was also variety dependent, particularly for higher alcohol acetate and ethyl ester of branched acids. This study highlights that varietal differences observed in Shiraz and Cabernet Sauvignon wines involve fermentation-derived compounds irrespective of the site (soil, climate, viticultural practices).



# Analytical developments from grape to wine, spirits : omics, chemometrics approaches...

## VI.SC 1

# STRATEGIES FOR SAMPLE PREPARATION AND DATA HANDLING IN GC-MS WINE APPLICATIONS

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**Keywords:** data mining, GCMS, sample preparation, untargeted analysis

It is often said that wine is a complex matrix and the chemical analysis of wine with the thousands of compounds detected and often measured is proof. New technologies can assist not only in separating and identifying wine compounds, but also in providing information about the sample as a whole.

Information-rich techniques can offer a fingerprint of a sample (untargeted analysis), a comprehensive view of its chemical composition. Applying statistical analysis directly to the raw data can significantly reduce the number of compounds to be identified to the ones relevant to a particular scientific question. More data can equal more information, but also more noise for the subsequent statistical handling.

Therefore, strategies to reduce the some of the data can already be applied at the chemical analysis stage without loss of information.

Using GCMS as analysis tool, an experiment was designed to evaluate on one hand different sample preparation methods, and on the other hand data handling strategies for the results. Twenty-six commercial wines from three cultivars (Chenin Blanc, Chardonnay, Sauvignon Blanc) and two winemaking styles (with and without wood contact) were subjected to three types of sample preparation (liquid/liquid extraction with three solvents, SPE on two stationary phases, HS-SPME on four fibres) before injection into GCMS. The various chemistries and polarities of the extraction solvents and stationary phases used resulted in different types of compounds being extracted from the wines.

The TIC data was exported as a continuous signal (the chromatogram itself), as integrated peaks identified by their RTs, and as a (RT<sub>m/z</sub>, abundance) matrix. Each type of data was submitted to PCA to underscore any natural grouping in the data. OPLS-DA and S-plots were subsequently used to determine the signals associated to cultivar discrimination and style. The raw data was revisited, and MS spectra extracted for the signals of interest, leading to the identification of the drivers (ions/compounds) for cultivars and style.

The strategies for sample preparation and data extraction were evaluated based on their feasibility and potential for data mining. Additionally, this type of work can be of further use as a basis for developing screening or targeted analyses, based on the groups of analytes extracted during various sample preparation procedures.

## VI.SC 2

## DEVELOPMENT OF A LC-FTMS METHOD TO QUANTIFY NATURAL SWEETENERS IN RED WINES

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**Keywords:** Orbitrap, method validation, wine, sweetness

The quality of a wine is largely related to the balance between its sourness, bitterness and sweetness. Recently, molecules coming from grapes have been showed to notably contribute to sweet taste of dry wines. To study the viticultural and oenological parameters likely to affect their concentration, their quantification appears of high interest and subsequently requires powerful analytical techniques.

Therefore, a new method using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) was developed and validated to quantify epi-DPA-3'-O- $\beta$ -glucopyranoside acid (epi-DPA-G) and astilbin, sweet molecules identified in wine. Three gradients were tested on five different C18 columns (Hypersil Gold, HSS T3, BEH, Synchronis and Kinetex).

The finest results were obtained upon using Hypersil Gold C18 and a gradient elution composed of 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile. Satisfactory linearity with correlative coefficient ( $r^2$ ) higher than 0.995 was achieved for both compounds with recoveries higher than 89 %. Good sensitivity ( $\text{LOD} \leq 7 \mu\text{g L}^{-1}$ ) and repeatability ( $\text{RSD} \leq 3\%$ ) were obtained.

The developed method was applied to screen epi-DPA-G and astilbin in red wines coming from several vintages over one century. Both compounds have been detected in all wines, at concentrations varying from 1.4 to 14.7  $\text{mg L}^{-1}$  for epi-DPA-G and from 0.5 to 32.2  $\text{mg L}^{-1}$  for astilbin. These results demonstrate the reliability of the developed method to quantify epi-DPA-G and astilbin in wine and suggest their oenological interest. Moreover, the method was used to study the influence of various winemaking parameters on epi-DPA-G and astilbin concentrations. The results opened promising perspectives for a better monitoring of extraction during vatting.

### VI.SC 3

## DEVELOPMENT OF A NEW METHOD TO UNDERSTAND HEADSPACE AROMA DISTRIBUTION AND EXPLORE THE PRE-SENSORY LEVEL IN PERCEPTIVE INTERACTIONS INVOLVED IN RED WINE FRUITY AROMA EXPRESSION

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**Keywords:** Analytical method development, Red wine, Sensory analysis, Perceptive interactions

A part, at least, of red wines fruity expression may be explained by perceptive interactions involving particularly various substituted ethyl esters and acetates present at concentration far below their olfactory threshold, specifically thanks to synergistic effects.

Wine sensory perception is directly linked to the stimulation of the taster at the level of olfactory epithelium by volatiles. These compounds are liberated from the matrix to the atmosphere, and will then be smelt. From a physico-chemical point of view, these volatiles ability to be released may be evaluated by their partition coefficients, which correspond to the volatile concentration ratio between the liquid and gas phase. Our goal is, through these coefficients determination, to assess if volatile matrix composition is able to impact the volatility of some compounds, and then explain sensory perception, i.e. to evaluate what is called the pre-sensorial level impact.

Up to our works, various experimental methods have already been developed to determine gas-liquid partition coefficients, but were not adapted to red wines fruity aromatic expression context. Recently, we have developed a new method coupling the low-pressure and static headspace gas chromatography to a mass spectrometry (LP-HS-GC-MS) in order to calculate simultaneously main esters partition coefficients, and that, at their wine concentrations.

This method of partition coefficients determination was used to study potential modifications of headspace aroma distribution and was applied to understand various perceptive interactions previously described by our team. Results revealed that pre-sensory effects may explain the effects observed during sensory analysis. For example, the presence of dimethyl sulfide led to an increase of esters partition coefficients, and therefore their concentration in the headspace what was correlated to the enhancement of the blackberry-fruit notes observed concomitantly. Furthermore, addition of malolactic fermentation by-products (as diacetyl, acetic acid, g-butyrolactone and acetoin) led to a decrease of esters partition coefficients, and thus of their concentration in the headspace, what may explain partly the masking effect of these compounds on fruity notes perception.





**Sensory properties,  
psychophysics,  
experimental economy,  
connections with  
neurosciences**

## STUDY OF THE AROMATIC OXIDATION MARKERS OF TEMPRANILLO LONG AGED WINES

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**Keywords:** Sensory analysis, Odour threshold, Tempranillo, GC-MS

The aromatic quality of wines after a long aging period in bottle is one of key points for oenologists. The objective of this work is to determine the main representative aromatic compounds found in long aged wines from D.O.Ca. Rioja. This study was made by 32 wines from 1971 to 2010 vintages. Sotolon, acetaldehyde, phenylacetaldehyde, 1,1,6-trimethyl-1,2-dihydronaphtalene (TDN),  $\beta$ -damascenone,  $\gamma$ -decalactone and  $\gamma$ -dodecalactone were determined as the most important oxidation markers by GC-MS analysis. Moreover, sensory analysis using triangular tests were performed from wines with and without the addition of the mentioned compounds. Four different concentrations of each odorant were added, as individual compounds and as mixtures. The additions were ranged from values close to the reference odour thresholds up to high level concentrations. The most identified aroma was sotolon, which is commonly associated to curry and coffee liqueur aromatic notes. Other oxidative compounds were easily detected by panellists, such as  $\gamma$ -decalactone (peach compote),  $\gamma$ -dodecalactone (ripe fruit). The mixtures of the odorants were most easily detected than the individual compounds. It should be noted that acetaldehyde and phenylacetaldehyde were rarely perceived and distinguished.

*Acknowledgments: The authors would like to acknowledge the Ministerio de Economía, Industria and Competitividad and the Centro for the Desarrollo Tecnológico Industrial (CDTI) for their financial support, Proyecto VINySOST Programa Estratégico de Consorcios de Investigación Empresarial Nacional 2014 (CIEN) and Rioja Alta S.A. for the wine samples and its contribution.*

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**Keywords:** astringency, subqualities, Sangiovese, aging

One of the main sensory characteristics of red wine is astringency, which can be defined as drying, puckering and roughing of the oral cavity after the exposure to tannin-rich wines. Tannins are the main responsible for the intensity of the sensation as well for the qualitative aspects of astringency. However, the total intensity of the sensation is not sufficient to fully characterize red wine astringency. Thirty-three different subqualities (Gawel *et al.* 2001) had been generated to describe the complexity of this multi-perceptual phenomenon, which includes both tastes, tactile, and flavor sensations. So, how to feel tannins during tasting? In this study, we used a sensory method that combine the training for astringency subqualities with touch-standards and the CATA questions, usually applied in consumer science, to evaluate the astringency subqualities of different typologies of Sangiovese: commercial and experimental wines. Sangiovese wine represents a good model for the study of astringency because it is generally characterized by a high content of low and high molecular weight proanthocyanidins. Commercial wines differed for percentage of Sangiovese (80-100%) grapes used in winemaking and for designation (Toscana TS, Chianti Classico CH, Chianti Riserva CR, Morellino di Scansano MS). The astringency profile of wines changed as the percentage of Sangiovese increased. Positive subqualities as velvet, soft, mouthcoat, and rich highly characterized the Sangiovese wine belonging to TS and CR designations. Moreover, the astringency subqualities related to blending or wood aging, represented the drivers of quality of commercial Sangiovese wines. Therefore, four experimental wines (SANG1, SANG2, SANG3, SANG4) made with 100% Sangiovese grapes in different wineries of Tuscany were also used to evaluate the subqualities of Sangiovese wine. At 8<sup>th</sup> months post-harvest (8 mph) wines were mainly characterized by green (Cf=40-60%), dry (35%), and adhesive (35-55%) terms, indicating that Sangiovese wine tannins were excessively astringent and acid (green), causing a drying and sticking sensation in mouth. In order to follow the evolution of the astringency profile of Sangiovese during time, wines have been evaluated at 14-16-20 mph. The SANG1 wine at 14 and 16 mph was characterized by hard tannins, which at 20 mph turned to corduroy and rich subqualities. The SANG2 wine at 14 and 16 mph was felt as satin and silk, while at 20 mph became rich, soft and mouthcoat. The SANG3 wine was silk, corduroy and persistent after 14-16 mph, and velvet and full-body after 20 mph. The SANG4 was velvet and grainy at 14<sup>th</sup> mph, rich and soft at 16<sup>th</sup> mph, and full-body, mouthcoat and persistent after 20 months. Finally, the astringency profile of Sangiovese wine has changed from an unripe astringency towards rich, full-body and mouthcoating sensations during aging. By means of the described sensory method, a detailed evaluation of the astringency profile of Sangiovese was made, and the evolution of the qualitative features of Sangiovese tannins during aging has been revealed for the first time.

## CROSSED APPROACHES TO EXPERIMENTAL ECONOMICS AND SENSORY ANALYSIS REGARDING NOBLE ROT SWEET WINES PERCEPTION

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**Keywords:** sensory perception, experimental economy, noble rot sweet wines, consumers

Noble rot sweet wines are reputed wines, traditionally elaborated according to a singular vinification process involving the harvesting of overripe grapes under the action of the ascomycete fungus *Botrytis cinerea*. These exceptional wines for the richness of their aromatic palette, evoking honey, dried fruits and citrus nuances often present a strong aging potential. Thus, several research works have contributed for the past 10 years to characterize their typical aromas and identify key volatile compounds as well as the parameters of their formation. However, although having high reputation and still being considered as high quality wines, they are facing a consumer crisis for the past 15 years.

Experiments have also been conducted to deepen the links between their composition (aromatic component, sugar content) and their sensory perception by an expert panel and a panel consumers (130 persons) in a context of experimental economics studies. In details, sixteen wines from various Bordeaux sweet wines denominations of appellation origin from 2015 vintage were initially submitted to a panel of professionals, oenologists and researchers to assess their typicality level (aromatic component, complexity, balance sweetness / acidity). This preliminary study has allowed to retain 4 wines representing 2 models of Bordeaux sweet wines, i.e. those considered with the best exemplary notes, in terms of aromatic profile and taste equilibrium, having a sugar content close to one hundred g/L and those considered as less typical, although fruity and having a sugar content close to 70 g/L. Chromatographic analysis on the main volatile markers of the aromatic component of the noble rot sweet wines confirmed a generally higher level of abundance of these compounds (lactones, furanones) in the wines with the best exemplary ratings. These 4 wines were then submitted to a panel of consumers. Among the highlights of this study, it is clear that consumers did not use as the first criterion the level of sugar concentration of wines but they recognized the originality of their aromatic component, including among other criteria naming information of controlled origin as well as on noble rot. This study encourage to involve consumers in order to refine the choices of winemakers in the definition of wine profiles.





# Posters



# Plant and Environment, Grape quality

I.P 1

## GREFFADAPT, A UNIQUE EXPERIMENTAL PLOT TO CHARACTERIZE A LARGE PANEL OF ROOTSTOCKS

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**Keywords:** rootstock, rootstock × scion interaction, conferred vigor, *Vitis berlandieri*

In the context of climate change, of social pressure to reduce the use of phytosanitary inputs, and of competition between agricultural areas and urbanization, it is crucial to imagine new wine-making systems in order to maintain the economic viability of viticulture and reduce its environmental impact. The plant material, i.e. variety and rootstock, is a relevant, environmentally-friendly way of adaptation in this context. In France, 31 grapevine rootstocks are registered, but only five of them represent more than 75% of vineyard areas. In addition to phylloxera resistance, rootstocks offer the opportunity to be adapted to limestone chlorosis and drought. Existing diversity represents a high adaptive potential, but is too little used. Taking advantages of the existing plant material is a valuable way to speed up the rootstock selection which is a long term process. Consequently, besides the selection of newly bred genotypes, characterizing existing rootstocks already used in foreign countries or available in germplasm collections, is a complementary strategy to allow a faster enlargement of the rootstock range available for winegrowers.

The experimental vineyard GreffAdapt was created to get quantitative data and update the agronomical characteristics of 55 rootstocks, with 30 rootstocks registered in France and 25 rootstocks used in other countries. These rootstocks were grafted with 5 scions in 3 blocks of 5 vines each. The scions, Cabernet-Sauvignon, Grenache, Pinot Noir, Syrah and Ugni-blanc, were chosen to be representative of the main vine growing areas in France and to be, as much as possible, genetically distant. The blocks were defined according to soil resistivity measurements. The statistical power of the experimental design was also calculated. Before planting, the genetic identity of each genotype was checked with 20 microsatellite markers and their sanitary status was analyzed with ELISA assays. Planting occurred in 2015, 2016 and was completed in 2017. Fresh weight of each plant was determined at grafting and the pruning weight of each vine has been recorded annually since the plantation. The plot was planted with a density of 6250 vines per Ha and is pruned in "Guyot mixte". The plot is located on the experimental vineyard of INRA Bordeaux and extends over an area of 0.84 Ha.

Phenotypic data were analyzed according to the parentage with the three main genetic backgrounds (*V. riparia*, *V. rupestris*, *V. berlandieri*). The significant relationship between these variables was discussed taking into account annual data sets. The first results showed that the range of conferred vigor among the panel of rootstocks was large enough to identify the diversity requested to fit the different production objectives in the French vineyards.

Overall, Greffadapt is a very unique experimental facility to speed up the selection of rootstocks and to analyze the relationship between conferred vigor and drought tolerance, two major selection criteria for rootstocks.

**I.P 2****LEAF METABOLOMIC CHANGES INDUCED IN A COMMON SCION ON DIFFERENT ROOT-SYSTEMS AND UNDER DIFFERENT IRRIGATION REGIMES**Misha KWASNIEWSKI

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**Keywords:** LC-MS, Grape, Physiology, Analysis

Up until recently rootstock impacts on scion physiology have been explained primarily by simple factors like rootstock vigor or root-system architecture, however there is a growing body of work showing more involved signaling and nuanced changes from rootstock/scion interaction. Untargeted analysis opens the possibilities of finding all metabolic shifts caused by these interactions, even the unexpected. Additionally, by inducing varied amounts of drought stress on the rootstock/scion systems it is possible to observe rootstock influence on scion plasticity under stress. Samples were taken from a research vineyard in Mount Vernon, MO containing own rooted Chambourcin as well as three rootstocks (1103P, 3309C and SO4) with three levels of irrigation (full compensation of evapotranspiration (ET), 50% of ET and unirrigated) which have been implemented since 2013. Leaf samples were taken at berry-set, veraison and harvest, and were cryogenically frozen in the field in 2017 and 2018. Samples were extracted and analyzed on a UPLC Q-ToF in both reverse phase and normal phase and in positive and negative ionization modes with chromatogram normalization and feature detection conducted using XCMS. Year played a large role in the observed variance generally causing all 12 treatments within a year to group. To better identify differences between treatments, comparisons were made within year with numerous features found to significantly segregate between treatments at a  $p < 0.01$  and a greater than 1.5 difference between treatments. For instance, in 2017 and 2018, 164 and 108 significant features were found respectively for these year's veraison samples, separating unirrigated and irrigated vines regardless of treatment. Differences were also found between rootstock regardless of irrigation regime, with numerous features identified as showing similar differences in both 2017 and 2018. This indicates some continuity between leaf metabolic differences on different rootstocks, even when year differences significantly impacted concentrations overall. Analyte identification and quantification is ongoing based on feature detection results and will be related to vine physiology measurements taken at sampling.

I.P.3

NEW FLAVANOLS GLYCOSIDES: FROM GRAPE TO WINE

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**Keywords:** flavanol glycosides, Tannin biosynthesis, Tannin extraction, Red wine

Monomeric and dimeric flavan-3-ol monohexosides were investigated in grape extracts (Syrah, Merlot, and Cabernet-Sauvignon Tannat, Alicante, Syrah, Merlot, and Grenache) and wines. 4'-O- $\beta$ -glucosyl-(+)-catechin and 7-O- $\beta$ -glucosyl-(+)-catechin standard were hemi synthesized by using the UGT71A15 glucosyl transferase from apple.

The quantitative evolution from grapes to their corresponding wines were followed by UHPLC-MRM for Syrah and Grenache varieties for the first time. 22 monomeric and dimeric mono- and diglycosides were determined with concentrations ranging from 0.7 nanograms to 0.700 micrograms per gram of grape tissue, and 0 to 60 micrograms per liter for wines. Some of these compounds may be used in the future as extraction markers specific for skins or seeds. These compounds may also be intermediates in proanthocyanidin synthesis during grape maturation as suggested in other plants.

## I.P.4

## PREHARVEST POMACE EXTRACT ELICITATION: INFLUENCE ON PHENOLIC COMPOSITION IN TEMPRANILLO VARIETY

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**Keywords:** grape marc extract, wine, elicitors, phenolic compounds

Phenolic compounds represent one of the most numerous, important and widely distributed groups of natural products in plant kingdom. In grape and wine research, these compounds are particularly important due to their effect on grape and wine quality as they play key roles in wine color and mouth- feel properties, as well as its aging potential and stability.

Based on these facts, several techniques have been applied to improve the phenolic content of grapes so, it has been proved that phenolic biosynthesis may be induced in response to different biotic and abiotic elicitors[1]. In this respect, elicitors are molecules able to stimulate plant defense mechanisms which include the activation of secondary biosynthetic pathways such as the one leading to the formation of phenolic compounds [2 ]

On the other hand, during winemaking process, grape pomace is the main solid organic waste from winery industries; resulting from the pressing and/or fermentation processes it is generated in large amounts in many parts of the world [3]. In the last years, it is estimated that 3% of grape pomace produced is reused for animal feed [4], other applications are as waste-based compost [5] and a possibility to improve thermal insulation on building construction [6] A new possibility of application could be the use of grape pomace as a possible elicitor in grape, in order to increase the biosynthesis of secondary metabolites. Authors such as Benouret *et al.* [7] have showed elicitor activity on suspension cultured cells of tobacco using a grape marc extract .

The aim of this work was to evaluate the effect of preharvest application in Tempranillo berries of a grape marc extract (product elaborated in Bodegas Familiares Matarromera) on the grape and wine phenolic composition. The grape treated with the pomace extract obtained higher concentrations of total anthocyanins and tannins, and the wines elaborated with grapes treated with the pomace extract also showed higher values of color intensity , total polyphenols, tannins and anthocyanins when they were compared to the control wines.

As a preliminary conclusion, it can be said that the exogenous application of a pomace extract could be an interesting alternative to be used as an ecological elicitor with two different objectives: to reduce the use of conventional agrochemicals and improve the phenolic composition of grapes and wines elaborated with them.

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I.P.5

USING WINE QUALITY INDICATORS TO EVALUATE LIFE CYCLE ENVIRONMENTAL IMPACTS OF WINE: ROOTSTOCK & IRRIGATION FIELD STUDY MEETS LIFE CYCLE ASSESSMENT.

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**Keywords:** life cycle assessment, rootstock, grape, wine quality

Consumers, farmers, industry, and policy makers are increasingly in need of information regarding the environmental impacts of the products they consume, produce, and manage. Life cycle assessment (LCA) is the predominate method for evaluating the full environmental impacts of a product, process, or service. LCA typically normalizes environmental impacts to a weight-based functional unit. However, this is arguably inadequate for many food products, and, in particular wines, which consumers tend to value for quality-based indicators over simply quantitative-based indicators. In this study we utilize data collected from a field trial investigating the impact of rootstock selection and irrigation rates in relation to yield and a variety of quality indicators, i.e., sugar content, titratable acidity,  $\beta$ -damascenone, and  $\beta$ -linalool concentrations. Using the results of the field trial as well as data on the various inputs to the grape and wine production process, this study estimated the global warming, acidification, and eutrophication potentials for Chambourcin grapes grown in Missouri, United States on own-rooted and grafted  $SO_4$  rootstock irrigated and not irrigated. The production process, or boundary of the LCA, includes: production and application of pesticides, field preparation, tillage, harvest, transportation, and vinification of harvested grapes. Note that fertilizers were not applied to the vineyards over this study period as the nutrient levels were sufficiently present; typically, nitrogenous fertilizers are a considerable contribution to the global warming potential estimated in other LCA studies of wine. Environmental impacts per kg grape and per liter wine were estimated. In addition, "efficiencies" were calculated for each quality indicator; efficiency is defined as 'unit of quality indicator' present per unit of environmental impact generated, e.g., degrees Brix per kilogram of  $CO_2$ -equivalent. Despite significant differences in yield, with irrigated treatments higher than unirrigated, preliminary results indicate little difference in environmental impact across treatments on a per kg basis. If we also consider quality indicators the "more sustainable" choice is less clear as reductions in yield are offset by increased concentrations of quality indicators. This presentation will explore the trade-offs using yield and quality indicators for LCA.

## I.P.6

## VITICULTURAL AND OENOLOGICAL MINIMIZATION STRATEGY FOR 1,1,6-TRIMETHYL-1,2-DIHYDRONAPHTHALENE (TDN) CAUSING PETROL OFF-FLAVOR IN RIESLING WINES

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**Keywords:** climate change, petrol off-flavor, Riesling wine, minimization strategy

1,1,6-trimethyl-1,2-dihydronaphtelene (TDN) evokes the odor of "petrol" in wine, especially in the variety Riesling. Increasing UV-radiation due to climate change intensifies formation of carotenoids in the berry skins and an increase of TDN-precursors [1]. Exploring new viticultural and oenological strategies to limit TDN formation in the future requires precise knowledge of TDN thresholds in different matrices.

The highly hydrophobic character of TDN leads to an unusual volatilisation behaviour, as thresholds of 2-3 µg/L hardly vary among matrices such as water, model wines and white wine [2]. While wine experts show an acquired appreciation for some petrol-notes in context of ripe or aged Riesling wines, most consumer don't like this foreign odor in wine at all.

This raises the question of a TDN minimisation strategy, especially since climate change enhanced TDN formation in general. The choice of rootstocks and Riesling clones showed in three vintages significant impact, however densely packed clusters yielding less TDN, are contradictory to phytosanitary aspects, favouring loose clusters for fast drying after precipitation. Varying timing and extent of defoliation decreased bound TDN precursors by a factor of 2-3 versus the untreated control. Sensory analysis showed enhanced petrol-notes in excessive defoliation, although this stimulated more intense fruity and floral attributes.

Press fractionation reveals two opposite effects: Higher pressure yields more TDN precursors, but lower pH in free-run and the first press fraction enhances TDN release by acid hydrolysis. Even a slight pH increase due to malolactic fermentation lowers the sensory perceptible free TDN. The strongest modulation was observed due to storage temperatures over 6 month, with 7°C maintaining the wines below detection thresholds, while 25°C yielded a TDN concentration close to detection thresholds of consumers (13,6 µg/L; [2]).

[1]: Winterhalter, P.; Goek, R., (2013), *ACS Symp. Series (1134)*, 125-137.

[2]: Ziegler, M. *et al.* (2019) submitted in in revision, *Food Qual. & Pref.*

## I.P.7

## BERRY AND WINE METABOLOME PLASTICITY OF A COMMON SCION ON DIFFERENT ROOT SYSTEMS WHILE UNDER VARIED IRRIGATION REGIMES.

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**Keywords:** untargeted metabolomics, rootstock, irrigation, grape-derived volatiles

The role rootstocks play on mediating environmental stresses on *Vitis vinifera* grapevines have been well studied, however, the effect of rootstocks on the phylloxera tolerant hybrid grapevines and the quality of fruit have not been fully explored. We investigated the plasticity of three commercial rootstocks and own-rooted vines, under different irrigation regimes on wine and berry volatiles in an experimental vineyard in Mount Vernon, MO. The trial contained own rooted Chambourcin as well as three rootstocks (1103P, 3309C and SO4). Three levels of irrigation (full compensation of evapotranspiration (ET), 50% of ET and unirrigated) were implemented. We sampled berries at veraison and harvest in 2017 and 2018 as well as made wines on a per vine basis. Samples were analyzed by SPME-GCMS/MS initially using a metabolomics approach followed by confirmation of compound differences using authentic standards. An untargeted approach is more inclusive and better suited to understanding total plant response especially in under studied varieties like Chambourcin. We observed differences in free and bound volatiles in grapes as well as wine volatiles due to irrigation and rootstock treatments as well as an interaction between these variables. Underlying these population differences were a large number of feature differences ( $m/z$  by time). For instance, we found 352 significant features in harvest 2018 berry samples across treatments for free volatiles and 520 features that are defining the differences between 1103P and own-rooted berries independent of irrigation (as determined by XCMS software). Feature differences were then used to identify metabolites. Among our findings were that a large number of esters and C13 norisoprenoids such as  $\beta$ -damascenone, were significantly different between own-rooted and 1103P vines regardless of irrigation treatment. However, there were also identifiable differences within the same rootstock, such as in 1103P, where 795 features different between irrigation regimes informed our targeted analysis that were used to identify volatile compounds responsible for those features including TDN, Ionone and Ethyl nonanoate among others. Additionally, the quantitative data from the 2017 wine showed that many important volatile compounds including  $\beta$ -linalool,  $\beta$ -ionone, TDN and  $\beta$ -damascenone are impacted by the irrigation levels as well as the interaction between the rootstocks and the irrigation, defining up to 30% of variance found.

## I.P.8

## A GRAPEVINE STILBENE EXTRACT PRODUCED BY RESVERATROL OXIDATIVE COUPLING SHOWS HIGH ANTI-MICROBIAL ACTIVITY AGAINST *PLASMOPARA VITICOLA* AND *BOTRYTIS CINEREA*

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**Keywords:** *Vitis vinifera*, stilbene oligomers, hemisynthesis, antimicrobial activity

Grapevine stilbenes are a group of polyphenols produced in various parts of the plant and identified as phytoalexins. Consequently, they contribute to the plant defense mechanisms under stress conditions such as fungal attacks. In vineyard, diseases represent a serious threat for the plant health, decreasing yields and hence wine production and quality. Several studies showed that grapevine stilbene extracts have high antimicrobial activities and could represent alternative or complementary methods to chemical pesticides use [Gabaston *et al.*, 2017; Pezet *et al.*, 2003]. These studies also indicate that the most active compounds are the oligomeric stilbenes like tetramers [Schnee *et al.*, 2013].

However, grapevine extracts mainly contain (E)-resveratrol (monomer) and (E)- $\epsilon$ -viniferin (dimer), that exhibit a moderate effect against fungal pathogens. In consequence, we wanted to produce extracts enriched in oligomeric stilbenes. To do that, we proceeded to a hemisynthesis of stilbene derivatives from resveratrol by oxidative coupling using metals. Obtained compounds were identified by MS and NMR spectrometry. The whole reaction product extract and the separated pure derivatives antimicrobial activity was then tested *in vitro* against *Plasmopara viticola* and *Botrytis cinerea*, the responsible agents of downy mildew and gray mold, respectively. Results showed a high efficiency of the reaction extract against both pathogens, with IC50 highly inferior to those of resveratrol and grapevine cane extracts.

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I.P.9

## A STILBENE ENRICHED EXTRACT TO CONTROL DOWNY MILDEW IN GRAPEVINE

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**Keywords:** Vineyard, *Plasmopara viticola*, bioprotection, polyphenol extraction

**Context:** Nowadays, agriculture and especially viticulture need more than ever to limit phytosanitary inputs due to environmental impacts. Therefore it is essential to develop biobased alternatives to protect grapevine against pathogens. We propose to develop a stilbene oligomer enriched (SE) extract obtained from grapevine by-products to control downy mildew in vineyard.

**Methods:** SE extract is produced from a mixture of *Vitis vinifera* L. wood and roots (70/30; w/w). Stilbenes were extracted twice with ethanol or ethyl acetate at different percentages (85 or 100%). After its filtration, the extract was or not purified by liquid-liquid extraction. Once lyophilized, SE extract was analysed by liquid chromatography. Its antimicrobial activity was assessed against *Plasmopara viticola* on leaf disks, in greenhouse and, vineyard in a formulated way.

**Results:** In order to identify an efficient extraction method for stilbene oligomers enrichment, two eco-friendly solvents (ethanol and ethyl acetate) were used, at different percentages. A liquid-liquid purification was performed as a last step. Results highlight the efficiency of ethyl acetate to extract trimers and tetramers. Extraction process using firstly the solvent at 100% then at 85%, were particularly optimal to recover tetramers and a good yield. Liquid-liquid purification increased total stilbene content but slightly decreased yield. In order to improve the effectiveness of the extract as protectant in vineyard, a formulation, aiming to enhance stilbene solubility and dispersion, was developed.

The antimicrobial properties of SE extract were assessed on the oomycete *P. viticola*. A high efficiency of the extract was noted on leaf disks (IC50: 90 mg.L<sup>-1</sup> and IC100: 300 mg.L<sup>-1</sup>). On foliar cuttings at 300 mg.L<sup>-1</sup>, the extract displayed 40 and 50% of protection plus or minus formulation, respectively. The vineyard assay in 2018 was a failure due to early season rain, high mildew pressure, and the washout of SE extract.

**Conclusion:** An extract enriched in oligomeric stilbenes was produced from grapevine by-products using a green solvent (ethyl acetate). It showed very encouraging protection against downy mildew on leaf disks and greenhouse assays. Nevertheless, the disease control is not conclusive in vineyard. This last result prompted us to improve the formulation to develop a remaining product. Thanks to this work, we confirmed the ability of stilbene enriched extracts as promising alternatives to pesticides in vineyard.

## I.P.10

ANTI-VEGF BIOACTIVITY OF STILBENES FROM GRAPE CANE OF *VITIS* SPP

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Angiogenesis, which involves the formation of new capillary vessels from pre-existing ones plays a crucial role on cardiovascular diseases. The most pro-angiogenic factor involved in this process is the vascular endothelial growth factor (VEGF) (Ferrara, 2004) by binding to its receptor VEGFR-2, which is the main mediator of the proliferation, migration, survival and permeability process on endothelial cells (Cébe-Suarez, Zehnder-Fjällman & Ballmer-Hofer, 2006). Recently, it has been reported that VEGF is the key molecular target for certain polyphenol compounds (Moyle *et al.*, 2015). Resveratrol has been the unique stilbene compound evaluated for the inhibition of VEGF-induced VEGFR-2 activation, which showed a weak inhibitory effect (24 % of inhibition at 50  $\mu$ M) (Cerezo *et al.*, 2015). However, there is no information about the anti-VEGF activity of other stilbenes. The aim of this work is to evaluate the potential anti-VEGF effect of twelve different stilbene compounds present in food, including monomers, dimers and tetramers, on the inhibition of VEGF and their subsequent effect on the downstream signalling pathway (PLC $\gamma$ 1, Akt and eNOS) on endothelial cells. To measure the phosphorylated VEGFR-2 a PathScan Phospho-VEGFR-2 (Tyr1175) sandwich ELISA kit was used. The signaling pathway phosphorylation of PLC $\gamma$ 1, Akt and eNOS were measured by Western-blot assay.

Results show that astringin was the most active stilbene against the VEGF phosphorylation (IC<sub>50</sub> = 2.90  $\mu$ M) followed by pallidol (IC<sub>50</sub> = 4.42  $\mu$ M),  $\omega$ - and  $\epsilon$ -viniferin (IC<sub>50</sub> = 6.10  $\mu$ M and 18.84  $\mu$ M, respectively) and piceatannol (IC<sub>50</sub> = 37.70  $\mu$ M). Furthermore,  $\omega$ - and  $\epsilon$ -viniferin inhibited the phosphorylation of PLC $\gamma$ 1 while active eNOS. Therefore, these compounds present good potential for their future exploitation as anti-VEGF ingredients in foods and beverages.

## I.P.11

## BIOCHEMICAL DIVERSITY OF VITIS GENUS REVEALED FROM TRITERPENOID PROFILES OF THE LEAVES OF WILD AND DOMESTICATED GRAPEVINES

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**Keywords:** Profiling, Triterpenoids, Steroids, GC-MS/FID

A considerable diversity in the biosynthetic abilities of grapevine species is linked to its heterogeneity and polymorphism. The aim of the present study was the comparison of the composition of triterpenoids in leaves of ten grapevines: four wild grapevine crops used as progenitors or rootstocks of domesticated vines (*Vitis aestivalis* Michx., *Vitis labrusca* L., *Vitis riparia* Michx., *Vitis vinifera* subsp. *sylvestris* (Gmelin) Hegl); and six domesticated *Vitis vinifera* cultivars: Alvarinho, Cabernet Sauvignon, Gamay, Marselan, Mauzac and Merlot. Samples were collected from experimental field in Villenave d'Ornon, freeze-dried at -80°C, lyophilized, ground and extracted with diethyl ether. Extracts were chromatographically fractionated and analyzed by GC-MS/FID. Although the main profiles of steroids and triterpenoids were similar in all extracts, remarkable quantitative differences in the content of these compounds in *Vitis* spp. leaves were demonstrated. The total content of triterpenoids ranged from 1.0 mg/g of dry leaf weight in Merlot to 2.7 mg/g in Mauzac; it was lower in leaves of domesticated cultivars (approx. 1.7 mg/g) than in wild grapevines (approx. 2.1 mg/g). The total content of sterols ranged from 0.6 mg/g of leaf d.w. in Merlot to 1.7 mg/g in Marselan; it was generally higher and more uniform in wild grapevines. The total content of pentacyclic triterpenoids ranged from 0.36 mg/g of leaf d.w. in Merlot to 1.5 mg/g in *V. labrusca*, and it was much lower in the domesticated grapevines (approx. 0.7 mg/g) than in the wild species (approx. 1 mg/g). In the majority of the analyzed wild grapevines, lupeol was the predominant pentacyclic compound, whereas among the domesticated grapevines, it was prevailing only in two cultivars, Alvarinho and Gamay, whereas in Cabernet Sauvignon, Marselan and Merlot, taraxerol was predominating. Thus, the predominance of pentacyclic triterpenoids of ursane-, oleanane-, lupane- and friedooleanane (taraxerane)-type skeletons depended on the studied cultivar/variety. The obtained data supplement information about biochemical diversity of *Vitis* genus and allow species discrimination considering phylogenetic relationships confirmed by HCPC analysis. It can be concluded that differences between wild and cultivated grapevines in steroid and triterpenoid profiles may be connected to the domestication process. The obtained results suggest that the profile of these compounds may be an important phenotypic trait of *Vitis* plants.

**I.P.12****CONTROL OF GRAPEVINE TRUNK DISEASES: INFLUENCE OF THE CURRETAGE ON THE QUALITY OF ONE-YEAR-OLD WINE****Emilie BRUEZ<sup>1</sup>**Céline Cholet<sup>1</sup>, Cécile Thibon<sup>1</sup>, Pascaline Redon<sup>1</sup>, Soizic Lacampagne<sup>1</sup>, Tommaso Martignon<sup>2</sup>, Massimo Guidici<sup>1</sup>, Philippe Darriet<sup>1</sup>, Laurence Geny<sup>1</sup>

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**Keywords:** Curretage, esca, Sauvignon blanc, wine quality

Currently, the increase of woody diseases, and particularly the esca, is affecting all the world's vineyards. This cryptogrammic disease showed typical foliar symptoms and necroses in the wood of trunk and cordon. The sodium arsenite, the only chemical product authorized was banned in 2001. Today, there is any chemical or biocontrol product efficient against the esca. Researchers and winemakers try to use new practical approaches against the esca such as the curettage. This method consists on using a saw that remove the white-rot, a specific necrotic woody tissue, from the cordon and sometimes from the trunk. This technic permitted to keep safe the diseased vine and not replace it. After a year, did the vines produce grapes and if yes, what is the quality of the berries and wine? Since 2016, in a chateau from Bordeaux region, a curettage trial on Sauvignon blanc cultivar was done. This is a sensitive cultivar to esca. Plants that expressed in 2012 and 2013 esca-foliar symptoms were identified and they were marked. During the springtime 2014, curettage was done on some of these plants and the other diseased plants were also kept for the experiment. For the healthy vines, no typical esca-foliar symptoms were observed since 2012. The goal of this study was to (i) taste the one-year-old white wine from harvest of 2017 and 2018 and (ii) to compare the wine between the curated plants, diseased and healthy vines. The results showed for the first year of wine tasting, the results of smell and taste did not differ between the curated vines and the healthy ones. Over a short time of aging, the quality of the wines seemed not to be affected by the curettage of the diseased plant.

I.P.13

## CONTROL OF GRAPEVINE TRUNK DISEASES: INFLUENCE OF CURETTAGE ON GRAPEVINE RESILIENCE AND BERRY QUALITY OF THE SAUVIGNON VARIETY FROM THE BORDEAUX REGION

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**Keywords:** curettage, Esca, *Vitis vinifera L.*, berry quality

Over the last two decades, the increase of Grapevine Trunk Diseases (GTD), particularly of Esca, is observed in most of the world vineyards. Various factors such as, the current change climate, the evolution of cultural practices and the ban of chemical treatments to control these diseases, could explain this GTD increase. Among the solutions proposed to control Esca, curettage is a cultural practice that consists in removing, by surgery, the necrotic wood, *e.g.* amadou, in order to preserve the healthy part of the vine from being contaminated.

The aim is also to promote the vine to be resilient. To our knowledge, no scientific experiments have been done to study the consequences of curettage on vine fertility and berry quality. In 2014, Sauvignon vines from a plot of the Bordeaux region have been curretted and we presented here a synthesis of a 3-year survey, *i.e.* 2016, 2017, 2018. Esca-asymptomatic vines since 2014 ("control") were compared to (i) diseased vines (M) and (ii) vines, curretted in 2014, without expressing Esca-foliar symptoms from that year (C14).

The first results showed that even if the curretted vines (C14) keep a lower vigor and fertility than the "control" vines, the quality of their grape berries is comparable to that of the "control" vines, which is not the case for diseased vines (M). Over time, curettage seems to allow the vines to be resilient and to compensate for the detrimental impact caused by Esca on berry quality.

I.P.14

## EFFECT OF PLANT ELICITORS BASED ON AMINO ACID AND VITAMIN K ON WATER STRESS AND GRAPE QUALITY IN NORTH-EAST SPAIN VINEYARDS

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**Keywords:** Elicitors, amino acids, bio-stimulant, water stress

Plant elicitors are considered as a sustainable solution to apply on grapevines in order to favour nutrient assimilation, improve water efficiency and support plant defence against diseases. This stimulant activity may help to increase drought resistance in a climate change scenario, reduce contamination of soils with nutrient excess, as well as reducing pesticide use in viticulture.

During four growing seasons, from 2015 to 2018, two different formulations of plant elicitors (from animal and vegetal origin), based in vitamin K and amino acids, were applied on North-East Spain vineyards. For this, different cultivars (Tempranillo, Grenache and Syrah) and irrigation conditions were used. The treatments consisted of four foliar applications between "berries groat-sized" and "veraison" phenological stages, with one-week spray intervals. Physiological variables, nutrient levels in leaves and must quality parameters were measured. Results showed an improved resistance to water stress in the treated vines when vineyard was not irrigated, while no differences were observed in the irrigated vines. Photosynthetic activity was significantly higher in treated plots in 2016 and 2017 experiments. Regarding nutrient levels on leaves, Boron level was increased in one of the assays. However, levels of nutrients and micro-nutrients were, in general, similar in the treated and untreated plots. As for grape must quality parameters, few significant differences were detected between the basic enological parameters. The content on Zn and Mn was improved in some cases as well as occurred for some aromatic compounds (aldehydes, terpenes and C6 compounds). Significant differences on the amino acids profile were observed in some of the studied conditions. The use of plant elicitors may represent a suitable solution to improve water efficiency, plant health and grape quality, although further research is needed to reveal factors that can improve their effects throughout foliar application.

*Acknowledgements. The authors would like to acknowledge for their financial support on the VID-ACITV Project (Ref. RTC-2015-3516-2) and also thank AGROSTOCK S.A. and MENADIONA S.L.*

I.P.15

## EVALUATION OF CHENIN BLANC WINES FROM SIX DIFFERENT TRELLISING SYSTEMS USING HRMS AND CATA

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**Keywords:** Chenin Blanc, trellis system, CATA, HRMS

Vineyard architecture is – if not impossible – then at least difficult to change once established. When planning a new vineyard, consideration must be given to a variety of factors that might influence the result.

The experiment took place over two seasons on one small block planted the same year with the same type of Chenin Blanc vines (clones and rootstocks). The six different trellising systems used are Lyre, T-Frame, Santorini, Ballerina, Smart-Dyson, and échalas (known locally as 'stalk by paaltjies'). The grapes were harvested at the same sugar level to avoid issues related to the differences in alcohol levels at the sensory evaluation stage. Winemaking was done in triplicate the same way for all treatments and included two months on the lees and cold stabilization, but no filtration.

Three months after bottling, the wines were subjected to chemical and sensory evaluation. The sensory evaluation was done by both industry experts and the in-house analytical panel. CATA was used with a comprehensive list of Chenin Blanc descriptors for aroma and a simplified rating (out of three) for taste and mouthfeel. The industry experts were also asked to evaluate wine quality on a 20-point scale. The wines were fingerprinted by HRMS.

The results indicated that the differences between the treatments were based on the taste and mouthfeel rather than aroma, and were consistent between the two panels used and for the two harvests. Aroma profiles could not be used to distinguish between all treatments, and in the two years different treatment groupings were observed. Most interestingly, the HRMS results subjected to PCA produced groupings based on the treatments for both years.

I.P.16

## GENOTYPING BY SEQUENCING AND GENETIC MAPPING OF PHENOLOGY-RELEVANT, BERRY QUALITY AND AROMATIC POTENTIAL TRAITS FROM TWO GRAPEVINE MAPPING POPULATIONS

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**Keywords:** Berry quality, Genotyping by sequencing, Phenology, QTL detection

Renewed grapevine breeding efforts are needed to meet the challenge of the ongoing climate change and the societal demand for low input viticulture. Our program aims to generate new cultivars that will meet these two goals. As a part of this program, two F1 intra-vinifera mapping populations were generated in order to identify molecular markers linked to phenology, berry quality, aromatic potential and Bordeaux wine typicality. The first generated F1 mapping population was obtained by crossing two red cultivars: Petit Verdot (PV) and Cabernet Franc (CF). Cabernet Franc cultivar is one of the progenitors of Cabernet-Sauvignon and Merlot, two emblematic premium red cultivars of Bordeaux vineyards. Petit Verdot cultivar is known for the complexity of its wine and its late ripening trait. Five hundred sixty plants were obtained from 1500 seeds. A set of 200 individuals was randomly selected for genotyping by sequencing (GBS) analyses, subsequent genetic map construction and quantitative trait loci (QTL) detection. A second F1 population from white grapevines was produced by crossing Ugni Blanc (UB) and Sauvignon Blanc (SB). Four hundred sixty-seven plants were obtained from 1300 seeds. A set of 200 individuals was also randomly selected to realize genotyping by sequencing (GBS) analyses. GBS libraries of these two populations were prepared with the help of the GPTR genotyping platform (UMR AGAP, Montpellier, France). The sequencing by Illumina HiSeq 3000 was performed on GET-plage platform (Toulouse, France) and the construction of the maps is in progress. After bioinformatic treatments, genome-wide association studies will be performed on the SNPs data sets with the aim to identify QTLs related with traits of interest (phenology of the plants, primary and secondary metabolites associated with Bordeaux wine typicality). The molecular markers identified in this project will be useful for marker-assisted selection and future breeding programs in grapevine. The project will bring to the wine industry the basis of the genetic determinism of some traits of interest for the production of wines with emblematic Bordeaux typicality.

*Acknowledgments:* We thank the CIVB for the financial support to the NEWVINE project N° 901 and to the GENOGRAPE project n°1801053/54

I.P.17

## GRAPES IN BELARUS: ECOLOGICAL ASPECT

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**Keywords:** grape, Brest region, cultivars, index of maturity

Belarus refers to the northern zone of viticulture. The history of grape introduction in region begins in the 13th century and since 1935 started a scientific study and testing of grape varieties. Now national ampelographic collection includes about 300 varieties. Ten varieties are listed in the national register and recommended for cultivation in the territory of Belarus: Agat donskoi, Krasa Severa, Kosmos, Kosmonavt, Alfa, Bianka Kristal and Marechal Foch. All these grape varieties were recommended for cultivation in the Brest region, which is primarily due to geographical location (southwest) and optimal climatic conditions.

The climate of the Brest region is moderately continental, accompanied by mild winters and moderately warm summers. The average January temperature is  $-2.6^{\circ}\text{C}$ , the warmest month (July)  $+19.3^{\circ}\text{C}$ ; the average annual  $+8.2^{\circ}\text{C}$ . The duration of the warm period in Brest has 258–260 days, and frost-free period – 155–175 days. The minimum amount of active temperatures varied between 2400–2600 $^{\circ}\text{C}$  for the Brest region.

The average annual amount of sunny hours for Brest reaches 1822, which is sufficient for the full maturation of grape varieties with a short growing period (the required minimum is 1300h). According to the long-term data, the value of the heliothermal Bran index in the Brest approaches 4.5, whereas the necessary minimum for the grapes cultivation is 2.6.

In amateur culture, grapes can be successfully cultivated everywhere in Belarus, taking into account the choice of assortment and planting site. Industrial cultivation of grapes was carried out only in the Brest region of Belarus. The total area of the vineyard is 70 hectares and the variety Alpha (*Vitis labrusca* × *Vitis riparia*) is predominantly grown.

In our study, the maturity index was calculated as the sugars to titratable acid ratio. Maturity index in this study ranged from 14.37 (red grapes) to 31.91 (white grapes). This important parameter can be increased using biostimulants with natural origin. After grape treatment of epibrassinolide (10–6%) the maturity index increases by 34.1% due to a decrease in titrated acidity (18.9%) and an increase in the sugar content (21.5%).

Thus, the Brest region is very promising for the expansion of viticulture with elements of organic farming.

**I.P.18****IMPACT OF GRAPE TEMPERATURE INCREASE ON THE BORDEAUX WINE QUALITY.****Céline CHOLET<sup>1</sup>**Cécile Thibon<sup>1</sup>, Alexandre Pons<sup>1</sup>, Julie Drappier<sup>1</sup>, Marie Andre<sup>1</sup>, Michael Jourdes<sup>1</sup>, Pascaline Redon<sup>1</sup>,  
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**Keywords:** climate change, quality, temperature, wine

Climate change induces more or less important changes in rainfall, lighting and temperature. These parameters are essential in maturation process, and temperature seem to be play a particularly role in grape-berry quality. But, the effect on Bordeaux wine quality is less well known. One of the goals of the HEAT BERRY project was to understand the impact of high temperature on grape berry metabolism and their effects on wine composition.

A passive heating system made of polycarbonate screens has been set up at the Cabernet Sauvignon and Sauvignon blanc vineyard in Bordeaux area to increase the berry temperature of 1–3°C between fruit set and harvest. 10 kg of berry of each treatment (Heated and Control non heated) were harvested to make wine by microvinification process. Quality of wines were evaluated by sensory and chemical analyses after 1 and 2 years of aged.

The effects of the temperature increase depends of the variety. In red wine, increase of temperature change principally the phenolic structure but not significantly odorous markers.

For white wines, some differences were observed on chemical odorous markers aromatic, but not difference was noticed for the sensory analysis even after aging.

I.P.19

LEAF REMOVAL DURING VINE VEGETATIVE CYCLE INFLUENCE PHENOLIC AND POLYSACCHARIDE COMPOSITIONS OF GRAPES AND WINES

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**Keywords:** Leaf removal, phenolic compounds, polysaccharides, astringency

The consequences of global climate change are affecting the grape maturation process and, consequently, the grape phenolic quality at harvest time. As a result, wines show an unbalance phenolic composition which results in defects on its color and astringency characteristics. Astringency is mainly related to the salivary protein precipitation because of the interaction with wine flavanols. However, the different flavanol structures (catechins, gallo catechins, galloylated derivatives) show different abilities for interacting with salivary proteins and, therefore, they show different astringent characteristics (Ferrer-Gallego *et al*, 2015). Moreover, wine polysaccharides have been described as inhibitors of those interactions and may hence lead to astringency modulation, although it depends on polysaccharide structure (Brandão *et al*; 2017). Flavanols, among other phenolic compounds, can also affect wine color since they have been reported as possible anthocyanin copigments and they are involved in several reactions that lead to different anthocyanin derivative pigments. It has been reported that grapevine vigor is highly correlated to grape anthocyanin and flavanol composition (García-Estévez *et al.*, 2017), so changes in the vine canopy during grapevine vegetative cycle could affect the phenolic composition of grapes and, therefore, that of wines. The main objective of this work was to assess the effect of leaf removal in different stages during grapevine vegetative cycle (pre-flowering stage or berry-set stage) on the detailed phenolic and polysaccharide compositions of grapes collected at veraison and at harvest time. Moreover, the phenolic composition and the sensory properties, *i.e.* color and astringency, of the wines made from those grapes collected at harvest time have been determined at different stages of winemaking and aging. Results pointed out that leaf removal can affect the flavanol composition of both grapes and wines, not only from a quantitative point of view but also qualitatively, since it can affect the ratio between procyanidin and prodelphinidins and the percentage of galloylated flavanols. Moreover, polysaccharide composition of wines is also influenced by leaf removal depending on the stage of grapevine vegetative cycle when it was performed.

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I.P.20

**PHYSICAL AND BIOCHEMICAL CHARACTERIZATION OF GRAPE SKIN FROM CHAMPAGNE REGION IN RELATION TO *BOTRYTIS CINEREA* SUSCEPTIBILITY****Marie ANDRE**<sup>1,2</sup>SandraI SMAIL<sup>2</sup>, Audrey BARSACQ<sup>1</sup>, Laurence MERCIER<sup>2</sup>, Laurence GENY-DENIS<sup>1</sup>

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**Keywords:** skin ripening, thickness, porosity, *Botrytis cinerea*

Skin grape ripening is characterized by important structural and biochemical changes. As well as being the interface between the external environment and the grape's interior, berry skin is also the place of synthesis of numerous oenological compounds of interests. During grape ripening, the skin's thickness decreases, increasing its susceptibility to pathogens such as *Botrytis cinerea*. There are two different mechanisms that manage grape skin degradation: first, there is an increase of elasticity in the berry skin; then, there is a degradation of the parietal polysaccharide structure, increasing porosity.

In this work, the physical and biochemical characterization were monitored on skin throughout the berries' development to explain the increase of *Botrytis cinerea* susceptibility in the Champagne context. Skin thickness, water availability, relative humidity and exudates were measured in relation with *in vitro* *Botrytis cinerea* susceptibility tests for two varieties: *Vitis vinifera* cv. Meunier and Chardonnay in Champagne region during the 2018 vintage.

Our results show that the skin ripening process differs between varieties: in Chardonnay berries, water availability and elasticity measurements are positively correlated; while in Meunier grapes, elasticity is positively correlated with relative humidity and negatively correlated with *Botrytis cinerea* susceptibility.

These two varieties behave differently, Chardonnay skin evolution is close to previous studies on Cabernet sauvignon and Merlot, while Meunier skins present a more important elasticity stage.

These first results can partially explain the difference of *Botrytis cinerea* sensibility between varieties. The objective of this study is to elaborate a physical and biochemical profile of berry skin for each cultivar to better understand the susceptibility of the berry and anticipate its possible degradation.

## INFLUENCE OF CAP MANAGEMENT, SEED TREATMENT AND PH ON THE EXTRACTION OF POLYPHENOLS FROM SKINS AND SEEDS DURING FERMENTATION OF PINOT NOIR AND CABERNET SAUVIGNON

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**Keywords :** polyphenols, red wine making, extraction, LC-QToF-MS

Because of the importance of polyphenols to sensory characteristics of red wine and because of their different sources and structures, research in this area has been a perpetual interest. Grape and wine production techniques, including vine vigor, fruit shading, fruit maturity, cold soak, berry crushing and seed removal, are reported to influence the extraction dynamics of polyphenols from grape skins and grape seeds. The objective of this study was to find out to what extent different fermentation practices influence the extraction dynamics of polyphenols from skins and seeds. Pinot noir and Cabernet Sauvignon grapes were de-stemmed, crushed and processed at original and decreased pH. Fermentations were conducted at 28°C for 10 days in 100 L fermenters. Automated cap management was carried out by punch downs, air push, pump overs or a rotating spindle fermenter. Seeds were removed at day 5 when they had fallen to the bottom of the fermenters, were kept outside or milled and returned back into the fermenters. LC-QToF-MS analysis and data processing according to the aspects of the pertinent structural information revealed that skin polyphenols were extracted faster than polyphenols from seeds in both grape varieties. Seed polyphenols gained in concentration after a lag time of three days. Seed removal at day 5 decreased procyanidin gallate and also the proportion of stable polymeric pigments. The polyphenol profile of Pinot noir was more affected by seed removal than Cabernet Sauvignon underlining the particular role of seeds in Pinot noir. Color intensity and stability were found to be increased when seeds were milled emphasizing the relevance of seed polyphenols for color stability. However, 4-fold higher tannin content after milling doubts the reasonableness of this treatment and the cap management during red wine fermentation is therefore of utmost importance. The investigated practices of cap management affected seed polyphenol extraction more than the extraction of skin polyphenols. The rotating spindle fermenter yielded two times more seed polyphenols than the air push treatment. The combination of a heavy mechanical impact and a high pH favored seed over skin polyphenol extraction. Acidification of grapes at the onset of fermentation yielded a higher proportion of momomeric structures, due to acid-catalyzed cleavage of interflavan bonds, as well as a higher proportion of ethylene-linked structures, due to enhanced protonation of acetaldehyde.

## INCREASED TEMPERATURE AND ATMOSPHERIC CO<sub>2</sub> MODIFY GRAPE COMPOSITION OF FIVE TEMPRANILLO (*Vitis vinifera* L.) SOMATIC VARIANTS

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As a consequence of the current levels of greenhouse gas emissions, global climate conditions are expected to be altered in the coming years. The changes will affect, among others, both global mean surface temperature and atmospheric CO<sub>2</sub> levels, which are expected to increase (IPCC, 2014). The result of these changes is known as climate change. The consequences of these changes are already being studied in different fields but with especially interest in agriculture. Grapevine (*Vitis vinifera* L.) is one of the most important crops from cultural and economic perspective, and its response to future growing conditions is not completely understood yet. Moreover, the rich intravarietal variety opens the possibility of reducing the potential adverse effects of climate change in this crop and, more specifically, mitigating negative consequences in wine production (Carbonell-Bejerano et al., 2015).

The objective was to determine the effects of increased temperature and atmospheric CO<sub>2</sub> levels (both acting independently and combined) on grape composition of five somatic variants of Tempranillo (RJ43, CL306, T3, VN31 and 1084), with the aim of detecting potential interesting traits among them. Fruit-bearing cuttings of the five somatic variants were grown in temperature gradient greenhouses under two temperature regimes (ambient temperature and ambient temperature + 4°C) combined with two CO<sub>2</sub> levels (400 ppm and 700 ppm) and treatments were applied from fruit-set to maturity (around 22°Brix). The concentration of sugars (glucose and fructose), malic acid and amino acids in grapes were measured throughout berry development, as well as the levels anthocyanins at maturity.

Grape production was affected by climate change conditions, especially elevated temperature, and the magnitude of these alterations were different among somatic variants, the long cycle variants being more affected. High temperature favoured the degradation of malic acid and hastened sugar accumulation in grapes, having stronger effect in 1084. Moreover, high levels of CO<sub>2</sub> also increased the degradation of malic acid, particularly at maturity. Total anthocyanin levels at maturity differed among somatic variants but they were not strongly modified by the increase of temperature and CO<sub>2</sub> levels.

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## FOLIAR SYMPTOMS, VASCULAR LESIONS AND INCIDENCE AND CHARACTERIZATION OF THE FUNGAL PATHOGENS DETECTED IN DECLINED VINEYARDS FROM CASTILLA LA MANCHA REGION

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**Keywords** : Grapevine trunk diseases, *Vitis vinifera*, foliar symptoms, fungal diversity

Grapevine trunk diseases (GTDs) are currently considered one of the most important problems threatening vineyards and wine industry. GTDs includes several diseases which are caused by a wide range of different fungi. These pathogens invade plants mainly through pruning wounds and colonise woody parts of the plants causing a slow decline. Most of these diseases also produce leaf symptoms, which include chlorosis, necrosis, deformation, and stunting. Spain is one of the major wine producers worldwide and almost the 50% of cultivated vines are grown in Castilla-La Mancha region.

For this job, eight symptomatic vineyards of different varieties of red (Cabernet Sauvignon, Tempranillo and Bobal) and white cultivars (Airen and Macabeo) have been used. Initially, for each vineyard, all vines were classified in four stages of the disease according to their visual symptoms (asymptomatic = 0% of damage; initial < 25% of damage; medium 25%-50% of damage; advanced > 50% of damage). One plant of each stage was collected from each vineyard to correlate foliar symptoms observed with vascular symptoms observed. Cross sections were made on rootstocks, trunks and cordons of sampled vines. Vascular symptoms were evaluated and each cross section was analyzed by fungi isolation. Root isolations were made too. The obtained isolates were identified by their morphological characteristic.

Vascular lesions can be detected from all asymptomatic and symptomatic vines. A total of 1.216 fungi isolates were detected, 258 of them were identified as grapevine trunk pathogens. These pathogens were classified into five different groups. *Cylindrocarpum*-like anamorphs are by far the most frequent species detected in our study, representing a 52.71%. *Phaeoconiella chlamydospora*, *Botryosphaeria* spp., *Phaeoacremonium minimum* and *Fomitiporia mediterranea* were also isolated, presenting an incidence of 22.48%, 12.02%, 9.69% and 3.1% respectively. All these fungi have been previously reported in other wine producing regions worldwide.





# Grape and wine microorganisms : diversity and adaptation

## II.P.1

**BRETTANOMYCES BRUXELLENSIS, A HIGHLY GENETICALLY DIVERSE SPECIES:  
A FOCUS ON WINE STRAINS****Alice CIBRARIO**

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**Keywords:** *B. bruxellensis*, Genetic diversity, Dissemination, Adaptation

The yeast *Brettanomyces bruxellensis* is known by winemakers for the production of volatile phenols which confer notable wine off-flavours described as leathery, sweaty animal and medicinal. Its presence and the associated flavours have been reported in several wine areas, all over the world, especially in red wines. Researches have shown that this yeast is able to maintain itself and even grow at low pH, high ethanol content and in the presence of low carbon and nitrogen concentrations. Furthermore, some individuals are resistant to sulphur dioxide, the main antiseptic used in wine.

*B. bruxellensis* is also found in beer, cider, kombucha, kefir or bioethanol where it is not necessarily considered as a spoilage agent. This wide range of occupied niches, and this ability to cope with difficult and diverse environments, question about the species' genetic traits. Indeed, several studies have pointed out a relatively high level of intra-species genetic diversity. Furthermore, genome assembly comparisons revealed the coexistence of diploid and triploid populations. A recent study conducted on a large collection of strains isolated from various matrices and geographic origins confirmed, on a large scale, the presence of strains with different ploidy level. This raises the question of the possible advantages the triploid state could confer. The analysis of the complete genotype dataset highlighted several genetic groups, which are correlated with the ploidy level and the niche of origin. Strains in all groups produce volatile phenols, but two groups were shown to display outstanding tolerance to sulfur dioxide. A large part of this collection was collected in wineries in the Bordeaux area and from early 20th century to 2015 vintages. We took advantage of this to explore the genotypic diversity among wine strains. The coexistence of diploid and triploid individuals belonging to distinct genetic groups was observed at the region, winery and wine lot levels. Isolates displaying identical genotypes were retrieved from different wineries, often very close (less than 2 Km) but sometimes separated from hundreds of kilometers. These results provide new highlights on the route of dissemination of this species and confirms an important adaptation of specific strains to wine.

## II.P.2

INVESTIGATION OF THE CAPACITY OF *BRETTANOMYCES BRUXELLENSIS* STRAINS TO FORM BIOFILM

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**Keywords:** *Brettanomyces bruxellensis* strains, Biofilm, Genetic groups, Wine spoilage

Biofilm mode of life allows microorganisms to a better adaptation to environmental conditions and contribute to the persistence of pathogenic and spoilage microorganisms in the food industry. In the wine industry, one of the most famous spoilage microorganisms is the yeast *Brettanomyces bruxellensis*, which can be found at several steps in the winemaking process due to its resistance to multiple stress conditions. However, the ability to form biofilms is another potential resistance strategy, although it has not been much explored so far. In this study, we propose to characterize biofilm of some strains of *B. bruxellensis* by microscopic analyses, and to study the capacity of strains to adhere and form biofilms on different materials, in synthetic medium or in wine.

By microsatellite analysis, 65 isolates studied have been discriminated into 5 different genetic groups. Twelve strains, representative of the 5 genetic groups, were selected to characterize the capacity to adhere and to form biofilms on polystyrene surface. For all strains, biofilm structure formed has been visualized by confocal laser scanning microscopy. Results revealed different cell morphotypes depending on the genetic groups and the physiological states of the cells. To investigate more in depth the biofilm formation of *B. bruxellensis*, 2 strains belonging to two different genetic groups were selected: biofilm colonization steps were studied on different oenological supports (oak and stainless steel) and in synthetic medium or wine. By scanning electronic microscopy, we demonstrated the presence of a matrix covering the cells adhered on stainless steel, an essential compound of biofilm that prove the capacity of *Brettanomyces bruxellensis* to form biofilm.

## II.P.3

### VITIS SPECIES DO NOT DRIVE POPULATION STRUCTURE IN CANDIDA ZEMPLININA (SYNONYM STARMERELLA BACILLARIS) SPECIES

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**Keywords:** *Vitis vinifera*, *Vitis labrusca*, *Candida zemplinina*, *Starmerella bacillaris*

The genetic diversity of *Candida zemplinina* (synonym *Starmerella bacillaris*), high in wine environments, is shaped by geographical location. Most analyzed *C. zemplinina* strains, however, have been isolated from *Vitis vinifera* L., disregarding the existence of other worldwide distributed *Vitis* species used in winemaking. In this report, we address the impact of the *Vitis* species and geographic location on the genetic diversity of *C. zemplinina*. Microsatellite genotyping analysis was applied to two remarkable populations of *C. zemplinina* from Argentina and Portugal (Azores Archipelago), isolated from neighboring *V. vinifera* and *V. labrusca* vineyards. The study also included a large population of previously characterized worldwide isolated *C. zemplinina* strains. No genetic differentiation on the basis of the *Vitis* species was recorded, indicating that *C. zemplinina* populations from neighboring *V. vinifera* and *V. labrusca* vineyards are genetically homogeneous. Our results indicate that only geographic localization significantly shapes the genetic diversity of *C. zemplinina*.

## II.P.4

QUANTIFICATION OF *BOTRYTIS CINEREA* LACCASES IN MUSTS AND WINES BY TARGETED TANDEM MASS SPECTROMETRY

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**Keywords:** *Botrytis cinerea*, Laccases, LC-MRM, Quantification

*Botrytis cinerea* is a necrophytic fungal pathogen infecting grapevine. It produces extracellular enzymes named laccases that are responsible for must and wine phenolic compounds oxidation and browning reactions. Recently, characterisation of laccases secreted by three *B. cinerea* strains (BO510, VA612 and RM344) evidenced some important differences in enzymatic activity in the corresponding isolated laccases. Furthermore, a qualitative proteomic study revealed the production of a mixture of two laccase isoforms (laccase 2, encoded by BcLcc2 gene, and laccase 3 encoded by BcLcc7 gene) in the three strains cultures.

The objective of this work is to quantify both laccase isoforms secreted in *B. cinerea* cultures and in contaminated musts and wines using a targeted proteomic approach.

Laccases were produced in three *B. cinerea* cultures (BO510, VA612 and RM344 strains) and isolated by tangential ultrafiltration. Proteins were isolated from Chardonnay must spiked with laccases by TCA/Acetone precipitation. The presence of laccases in three naturally botrytized white wines was also investigated. Protein extracts were processed by filter-aided sample preparation and digested by trypsin. Tryptic peptides were concentrated before injection on QqQ Mass Spectrometer. Analysis of both laccase isoforms was performed by LC-MRM. Two specific peptides were used to quantify both types of laccases.

Analyses revealed differences in laccases concentrations in the three strains cultures. Laccase 2 was predominant in the three cultures. TCA/Acetone protein extraction of a white Chardonnay must provided a high laccase 2 recovery ( $110.36 \pm 32.44$  %). A lower laccase 3 recovery was observed ( $31.53 \pm 1.29$  %). Laccases 2 and 3 were both detected in the three bottled commercial botrytized white wines. Laccase 2 concentrations ranged from 1.13 to 2.45 mg/l. Laccase 3 concentrations were 1000 times lower with values under a  $\mu\text{gramm}$  per litter.

In conclusion, an extraction method and a quantitative analysis method of *B. cinerea* laccases was developed. For the first time, two laccase isoforms of *B. cinerea* were quantified in white must and in botrytized white wines.

## II.P.5

GENETIC DISTRIBUTION OF *BRETTANOMYCES BRUXELLENSIS* STRAINS ISOLATED FROM GREEK WINEMaria DIMOPOULOU\*<sup>1,2</sup>Isabelle MASNEUF-POMAREDE<sup>2,3</sup>, Warren ALBERTIN<sup>2,4</sup>

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**Keywords:** wine spoilage, *Brettanomyces bruxellensis*, microsatellite markers, sulphur dioxide

The *Brettanomyces bruxellensis* yeast species is one of the major sources of wine alteration. In fact *B. bruxellensis* can produce large quantities of volatile phenols, which impart to wine an unpleasant taste and odor, commonly described as "stable odors" or "horse sweat" and mask the fruity nuances of the red wines. The contamination of the spoilage yeast constitutes a significant economic loss for the wine industry especially for the wine aging in wooden barrels. According to the genetic distribution of the species, it seems that human related factors influence the population structure. In our study we examined the genetic distribution of *B. bruxellensis* strains isolated from Greek wine for the first time and we compared them with a selection of 1500 strains isolated from fermented beverages from all around the world. The genetic typing of the strains was realized with microsatellite markers analysis by using 12 pairs of primers specific for the species. The dendrogram was constructed using Bruvo's distance and Neighbor Joining (NJ) clustering. According to our results the species of *B. bruxellensis* is characterized by high intraspecific genetic diversity. Statistical analysis of the generated data highlighted that both matrix of isolation and geographical origin of the isolates contribute to the observed population structure. Our results confirmed the relationship between genetic groups and survival patterns in the presence of sulphur dioxide. More precisely we propose that intensive use of sulphite to control *B. bruxellensis*, resulted in the emergence of sulfite-resistant strains. The prediction of *B. bruxellensis* SO<sub>2</sub> sensitivity through genetic markers analysis will permit the wine-makers to adapt their control techniques, to better manage their wine quality in order to produce authentic wines that express the subtleties of their terroir.

## II.P.6

ADAPTIVE EVOLUTION TO IMPROVE ACID TOLERANCE IN *OENOCOCCUS OENI*

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**Keywords:** *Oenococcus oeni*, Adaptive evolution, Acid tolerance, Stress response

*Oenococcus oeni* is the main lactic acid bacteria (LAB) associated with malolactic fermentation (MLF) of wine. MLF plays an

important role in determining the final quality of wines. Nevertheless, in spite of the stressful conditions inherent to wine and especially due to the acidity MLF may be delayed. Improved bacteria tolerance to those abiotic stresses appear to be beneficial in increasing the efficiency of MLF.

This study aimed to use adaptive evolution to improve acid tolerance but also for a better understanding of the mechanisms involved in adaptation in *O. oeni*. The ATCC BAA-1163 strain was propagated in a temporally varying environment, which consists of gradual pH decrease. Beginning from pH 5.3, the adaptive evolution experiment was performed for approximately 450 generations reaching final pH value of 2.9.

Acido-resistance of each separated isolates was compared to the parental strain by a pH 1.9 stress challenge during 90 min and different physiological parameters were appreciated such as membrane permeability, intracellular pH and membrane fatty acid compositions. Finally, populations were sequenced to estimate high diversity in the whole population. Performance of malolactic fermentation of each isolates by direct inoculation in wine is in course.

## II.P.7

### CARBOHYDRATE PREFERENCES IN *BRETTANOMYCES BRUXELLENSIS*

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**Keywords:** *Brettanomyces bruxellensis*, Carbohydrate, Metabolism, diversity

*Brettanomyces bruxellensis*, which causes off-flavors described as sweaty animal, leather and medicinal due to the production of volatile phenols, is highly adapted to the winemaking process. It can be detected at almost every stage of winemaking, from alcoholic fermentation to bottling, which makes it one of the most dreaded wine spoilage microorganisms. This is an important issue in the wine industry as Brett spoilage leads to economic losses due to the rejection by the consumers. Thus, all actors in the sectors are looking forward to solutions to prevent the development of these yeasts. *B. bruxellensis* is also associated with other industrial fermentations such as beer, cider, kombucha (fermented tea), kefir, bioethanol and others. In those last cases, the desirability/undesirability of this yeast is unclear and still debated. Several studies revealed a high level of genetic diversity at intra-species level. Furthermore, a comparison of genome assemblies revealed the coexistence of diploid and triploid populations and interspecific hybridization events. This genetic diversity explains the different levels of tolerance to abiotic factors such as low pH and high sulphur dioxide or ethanol concentrations. On the contrary, whatever their genetic group or isolation substrate, the strains examined are able to grow on a high diversity of single carbon and nitrogen sources.

We investigated whether different strains representative of the species genetic diversity exhibited the same preferences when grown on substrate mixtures (glucose-fructose, glucose trehalose and glucose-galactose). This work showed different behaviors, suggesting more pronounced catabolic repression phenomena in triploid strains compared to diploid ones. The consequences for wine and other products fermentations are discussed.

## II.P.8

## CHARACTERIZATION AND OENOLOGICAL APPLICATION OF AN HIGH-PREFORMING STRAIN OF *OENOCOCCUS OENI* TO DRIVE MALOLACTIC FERMENTATION. A REVIEW ABOUT 15 YEARS OF RESEARCH AND WINEMAKING

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**Keywords:** FML, *Oenococcus oeni* , Limiting conditions

Malolactic fermentation (MLF) is a biological process fundamental to determine wine quality, however is frequently affected by troubles due to the difficult acclimation of lactic bacteria to wine conditions. In this work, we review 15 years of collaboration between oenologists and researchers to propose efficient solutions for the management of MLF.

The work started with the evaluation of the resistance of 10 *Oenococcus oeni* strains to wine limiting factors, in order to select a suitable strain for a reliable MLF in wines presenting difficult conditions. Resistance to low fermentation temperature, high SO<sub>2</sub> and/or ethanol concentration, and low pH were assayed in laboratory tests. A pool of the most resistant strains was tested in laboratory MLFs. At the end of fermentation, the dominant strains were identified by RAPD-PCR. One strain was found to be dominant in the majority of MLFs, under the most detrimental wine conditions. Therefore, it was chosen as selected strain for the inoculum of MLF trials in industrial wineries.

The effectiveness of the selected strain of *O. oeni* strain was confirmed in different vintages, through more than 50 MLFs carried out in different oenological realities. It accomplished MLF in wines having about 16% ethanol, pH low as 3.00 or more than 50 mg/L of SO<sub>2</sub>, and in fermentation temperatures below 17 °C.

Following the recent trends of wine technology, selected strains of *O. oeni* were involved in malolactic fermentation conducted by different approaches, focusing the attention on the simultaneous alcoholic and malolactic fermentations in grape must. Experimental winemaking takes place in different Italian wineries. A careful monitoring of evolution of MLFs and an extensive characterization of obtained wines ensures a comprehensive overview about the potentiality of different MLF's protocols to contribute to organoleptic profile of wines.

## II.P.9

# CHARACTERIZATION OF *BRETTANOMYCES BRUXELLENSIS* STRAINS: IS THERE ANY POSSIBLE LINK BETWEEN SURFACE COLONIZATION PROPERTIES AND GENETIC GROUP?

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**Keywords:** *Brettanomyces bruxellensis*, surface colonization, wine spoilage, genetic diversity

*Brettanomyces bruxellensis* is a serious source of concern for winemakers. The production of volatile phenols by the yeast species confers to wine unpleasant sensory characteristics which are unacceptable by the consumers and inevitably provoke economic loss for the wine industry. This ubiquitous yeast is able to adapt to all winemaking steps and to withstand various environmental conditions. Moreover, the ability of *B. bruxellensis* to adhere and colonize inert materials can be the cause of the yeast persistence in the cellars and thus recurrent wine spoilage.

In the present work, we selected eight strains representative of the genetic diversity of the species, to investigate the surface properties, biofilm formation ability and the factors which may affect the attachment of the yeast cells to surfaces. Furthermore we successfully developed and applied protocols in order to examine various biochemical and physicochemical traits putatively linked with the colonization properties of *B. bruxellensis* in wine related environment. Our results show that the bioadhesive capacity is strain-dependent and suggest a possible link between the physicochemical properties of the studied strains and their corresponding genetic group.

## II.P.10

## DEVELOPMENT OF A MALDI-TOF/MS METHOD FOR MONITORING GRAPE AND WINE MICROORGANISMS

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**Keywords:** wine yeast, wine bacteria, MALDI-TOF/MS identification, species

Conventional microbiological methods used to monitor yeasts and bacteria of grape and wine are based on Petri dish culture, microscopic observation, quantitative PCR or flow cytometry. These methods are useful for monitoring microbial populations during all fermentation stages and to avoid wine spoilage. For more complex analyzes requiring identification of microorganisms at the species level, only molecular biology methods based on PCR and sometimes sequencing PCR product can be used. However, these methods are fastidious, expensive, and require strong technical skills, which limit their use by most laboratories of oenological analysis. In another context, the medical sector, another method is commonly used to identify microbial species: MALDI-TOF/MS, Matrix-Assisted Laser Desorption Ionization – Time Of Flight/Mass Spectrometry. This method is fast, reliable, inexpensive and easy to use. Any yeast or bacteria can be identified at the species level from a single colony, and a large number of microbial colonies can be analyzed within a few hours. Recently, MALDI-TOF/MS has been adapted to identify microorganisms associated with fermented products such as beer. It is not yet used in the oenological sector because a major constraint is that each species of yeast or bacteria must be analyzed and recorded in a database of reference species prior utilization. The reliability of the method is highly dependent on this database and so far it does not exist for microorganisms of grapes and wine.

The aim of this study is to develop the MALDI-TOF/MS method to target yeasts and bacteria from grapes and wine. First, different culture and analysis conditions for oenological microorganisms have been tested and optimized. Then a specific database is currently in progress using a wide variety of oenological yeast and bacteria species obtained from the laboratory culture collection (CRBO, Biological Resources Center – Oenology). Finally, the first application of MALDI-TOF/MS for analyzing the microbial community of wine is reported.

## II.P.11

### DISTINGUISHING THE COMPLEX AROMA OF PORT WINE

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**Keywords:** Port wine, yeasts, volatile composition, gas chromatography

Port wine is a fortified wine produced in the Douro Appellation (Portugal) under very specific conditions resulting from natural and human factors. Its intrinsic aroma characteristics are modulated upon a network of factors, such as the terroir particularities, varieties and winemaking procedures that include a wide set of parameters, namely the yeast strains. Over the past three decades, targeted consistency in winemaking has led to the almost ubiquitous application of commercial *Saccharomyces* strains. Although the recent introduction of commercial non-*Saccharomyces* strains has resulted in improved complexity, the potential impact and diversity of native Douro yeast strains responsible for Port production have yet to be studied, meriting the present investigation looking at the impact towards grapes from different terroirs.

The aim of this research work was to make an in-depth study to comprehend the impact of the binomial “yeast strain versus terroir” on the potential aroma characteristics of Port wine produced from the *Touriga Nacional* variety. The strategy developed included the analysis of wine volatile composition, sensorial properties, and yeast population profiling through fermentation, permitting a comprehensive understanding of the impact of “terroir versus yeasts”. The wines were analyzed using an advanced multidimensional gas chromatography methodology (HS-SPME/GC×GC-ToFMS) tandem with ANOVA-simultaneous component analysis and hierarchical clustering analysis. Attention was principally focused on volatiles reported as exhibiting high level odor activity values in Port wines [1]. Several volatile components were determined distributed over the chemical families of acids, alcohols, aldehydes, terpenic compounds, esters, norisoprenoids, and volatile phenols.

This research reveals that native strains were detected in all the conditions analyzed, including those inoculated with commercial strains. Despite the significant contribution made by yeast strains, terroir had the greatest impact modulating Port wine aroma.

[1] Rogerson, F. S. S.; De Freitas, V. A. *Journal of Food Science*, 2002, 67(4), 1564–1569.

Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/GUI/00062/2019) through national funds and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020 and to the Project PORTO NÃO *SACCHAROMYCES* selection of non-*Saccharomyces* yeasts endogenous to Port Wine.

## II.P.12

DISTRIBUTION BETWEEN SO<sub>2</sub> RESISTANT *BRETTANOMYCES* STRAINS AND SENSITIVE STRAINSVincent RENOUF

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**Keywords:** *Brettanomyces*, Sulphur, Resistance, Test

*Brettanomyces bruxellensis* is still one of the most important problematic for the red wine producers. Researchers Isabelle Masneuf and Warren Albertin (Unité de recherche Oenologie, ISVV) have established differences in SO<sub>2</sub> resistance between *Brettanomyces* strains. They established that these differences are due to some genetic particularities. A genetic test has been developed to differentiate sensitive strains and resistant strains. We have used these molecular tests to follow the evolution of the strain's diversity in three different estates from the grapes until the bottling. We have also analyzed the strains isolated from the cellar, the equipment and in the air. We have called this analysis the TYP\Brett test. It identifies the strains which possess the triploidy conferring resistance to sulfur. TYP\Brett provides very interesting data: i) the majority of *Brettanomyces bruxellensis* strains present in the vineyard are sensitive to sulphites, ii) in the musts and after in the wines, the percentage of resistant strains varies according to the physicochemical parameters but also to the oeno-technical practices. Thus, this distribution varies according to the estates, iii) Isolated strains of the atmosphere, on the surface of containers or small material are triploid strains resistant to SO<sub>2</sub>. TYP\Brett is a very efficient tool to follow and to characterize the *Brettanomyces* populations in order to prevent the volatile phenols production. It is the first microbiological analysis combining the precision of genetic typing with a practical information on the action to be taken to control the development of cells and thus the wines alterations.

## II.P.13

# FERMENTATION KINETICS AND NITROGEN REQUIREMENTS OF DIFFERENT NON-SACCHAROMYCES YEAST OF OENOLOGICAL INTEREST

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**Keywords:** non-*Saccharomyces*, amino acids, ammonium, fermentation

The use of non-*Saccharomyces* species in winemaking is increasing, mostly due to their positive contribution to the final quality of wines. It was demonstrated that these yeasts produce some volatile compounds different from the ones produced by *Saccharomyces* strains. Therefore, there is the necessity to delve into the study of non-*Saccharomyces* yeasts to optimize their use in mixed sequential fermentations with *Saccharomyces*. In this work, we have analysed the fermentative capacity and nitrogen consumption of several non-*Saccharomyces* yeast species of oenological interest (*Torulaspota delbrueckii*, *Lachancea thermotolerans*, *Starterella bacillaris*, *Hanseniaspora uvarum* and *Metschnikowia pulcherrima*).

We studied the effect of three types of nitrogen sources in each yeast species. For this purpose, synthetic must has been used to obtain different nitrogen compositions: one with amino acids, one with ammonium and one with mixture of amino acids and ammonium. Fermentations were carried out by triplicates at 22°C and 120rpm. The fermentation kinetics, nitrogen consumption and yeast growth were followed over time.

The results showed that the nitrogen consumption and preferences, was species dependent. Moreover, the nitrogen composition of the must had a direct effect on their fermentation profiles. *St. bacillaris* and *T. delbrueckii* fermented better with organic nitrogen in the medium, while *H. uvarum* fermentation was favoured by the presence of ammonium as the only nitrogen source. On the other hand, *M. pulcherrima* strains were unable to finish the fermentation, leaving a high concentration of sugar and nitrogen in the medium. Therefore, this work shows that it is important to control the nitrogen availability in mixed sequential fermentations, as some nitrogen compounds may be depleted by non-*Saccharomyces* before inoculating *Saccharomyces*, impairing its growth, and the performance of the fermentation.

This work was part of the project NUTRIAROMA (IDI-20160102), funded by Productos Agrovin S.A. and CDTI (Spanish Ministry of Economy, Industry and Competitiveness).

**II.P.14****FOLIAR SYMPTOMS, VASCULAR LESIONS AND INCIDENCE AND CHARACTERIZATION OF THE FUNGAL PATHOGENS DETECTED IN DECLINED VINEYARDS FROM CASTILLA LA MACHA REGION****M. Rosario SALINAS**

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**Keywords:** Grapevine trunk diseases, *Vitis vinifera*, foliar symptoms, fungal diversity

Grapevine trunk diseases (GTDs) are currently considered one of the most important problems threatening vineyards and wine industry. GTDs includes several diseases which are caused by a wide range of different fungi. These pathogens invade plants mainly through pruning wounds and colonise woody parts of the plants causing a slow decline. Most of these diseases also produce leaf symptoms, which include chlorosis, necrosis, deformation, and stunting. Spain is one of the major vine producers worldwide and almost the 50% of cultivated vines are grown in Castilla-La Mancha region.

For this job, eight symptomatic vineyards of different varieties of red (Cabernet Sauvignon, Tempranillo and Bobal) and white cultivars (Airen and Macabeo) have been used. Initially, for each vineyard, all vines were classified in four stages of the disease according to their visual symptoms (asymptomatic = 0% of damage; initial < 25% of damage; medium 25%-50% of damage; advanced > 50% of damage). One plant of each stage was collected from each vineyard to correlate foliar symptoms observed with vascular symptoms observed. Cross sections were made on rootstocks, trunks and cordons of sampled vines. Vascular symptoms were evaluated and each cross section was analyzed by fungi isolation. Root isolations were made too. The obtained isolates were identified by their morphological characteristic.

Vascular lesions can be detected from all asymptomatic and symptomatic vines. A total of 1.216 fungi isolates were detected, 258 of them were identified as grapevine trunk pathogens. These pathogens were classified into five different groups. *Cylindrocarpon*-like anamorphs are by far the most frequent species detected in our study, representing a 52.71%. *Phaeoconiella chlamydospora*, *Botryosphaeria* spp., *Phaeoacremonium minimum* and *Fomitiporia mediterranea* were also isolated, presenting an incidence of 22.48%, 12.02%, 9.69% and 3.1% respectively. All these fungi have been previously reported in other wine producing regions worldwide.

II.P.15

GENETIC CHARACTERIZATION OF LEBANESE INDIGENOUS *SACCHAROMYCES CEREVISIAE* ISOLATES FROM MERWAH WHITE WINE

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**Keywords:** indigenous, *Saccharomyces cerevisiae*, Microsatellite, diversity

The use of local indigenous grapes varieties is based on the valorization of the local heritage. Lebanon has a rich heritage of indigenous grapes like "Merwah and "Obeidy". There is a little data about the microbial diversity of indigenous yeast associated with Lebanese grapes. In this work 202 *Saccharomyces cerevisiae* isolates were collected from eight must/wine samples of Merwah for 2 consecutive years (2016 and 2017) during spontaneous alcoholic fermentation at the Lebanese winery "Château Byblin", located at Wata el Jozz in the Mount of Lebanon (1400m). The strains were analyzed through a robust molecular method based on 12 microsatellites markers with the aim of gaining deeper knowledge on the genetic variability and population structure of Lebanon fermentative yeast. 194 isolates presenting less than three missing data, were kept for genetic analyses. Among these, 180 different genotypes of *Saccharomyces cerevisiae* were identified, thus revealing a high genetic diversity in the studied fermentations. The diversity index, calculated on the basis of the number of different genotypes, was high, and remained constant during the alcoholic fermentation (middle and end of alcoholic fermentation) and in the vineyard considered. To help the winemakers to select a starter culture from this population, a phenotypic analysis is currently carried out to evaluate the fermentative performances and sensorial impact of the selected strains.

## II.P.16

**IMPACT OF *OENOCOCCUS OENI* ON *BRETTANOMYCES BRUXELLENSIS* DEVELOPMENT IN WINE-LIKE MEDIUM AND WINE – COMPETITION FOR WINE CARBOHYDRATES.****Patricia BALLESTRA**

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**Keywords:** *Oenococcus oeni*, *Brettanomyces bruxellensis*, wine, competition

*Brettanomyces bruxellensis* is one of the most relevant spoilage microorganisms in wine. This yeast is responsible for production of volatile phenols that depreciate the organoleptic quality of wines conferring "stable", "horse sweat", or "leather" aromas. *B. bruxellensis* strains are able to survive on many surfaces in wineries such as fermentation tanks or aging barrels. They can contaminate wine at different stages of its elaboration and develop during winemaking. They are particularly well adapted to the physico-chemical conditions characterizing wines after the alcoholic fermentation (AF) and are able to use a wide range of residual substrates. Moreover, some strains are tolerant to SO<sub>2</sub> often used to prevent their development.

If the most effective method to control *B. bruxellensis* remains cellar hygiene, the risk of wine spoilage can be further minimised by performing malolactic fermentation (MLF) under controlled conditions. Some studies have reported that inoculation of wine with selected lactic acid bacteria to induce and accelerate MLF can be an effective tool to protect the wine from *B. bruxellensis* spoilage. The aim of our work was to extend such investigations. We studied the possible competition between *Oenococcus oeni* and *B. bruxellensis* for wine carbohydrates. Two pairs of strains were selected for the study.

First experiments were conducted in a wine-like medium containing low contents of glucose, fructose and trehalose. The results showed that *O. oeni* metabolic activity during and after MLF did not influence *B. bruxellensis* growth. If a competition for consumption of fructose and glucose was sometimes established between strains, it was not sufficient to inhibit *B. bruxellensis* development.

Yeasts consumed trehalose more quickly than bacteria and their growth was not impacted by *O. oeni* metabolism. In red wine, the addition of a selected *O. oeni* strain to promote MLF led to the reduction in the development of *B. bruxellensis* strains inoculated in wine. However, the inhibition was not maintained over time. Three weeks after the end of MLF, *B. bruxellensis* population and ethylphenols production reached values observed in absence of *O. oeni*. Trehalose was the only residual sugar consumed totally. Exploring other *O. oeni* strains for their ability at degrading trehalose could be an interesting and useful approach to limit *B. bruxellensis* development.

II.P.17

## INVESTIGATION OF ACID STRESS RESISTANCE IN *OENOCCOCUS OENI* BY ITRAQ QUANTITATIVE PROTEOMICS

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**Keywords:** *Oenococcus oeni*, acidity, stress, proteomic

*Oenococcus oeni* is the main lactic acid bacterium species responsible for malolactic fermentation (MLF) in wine. This is due to its ability to develop in the harsh conditions of wine, and particularly to survive and proliferate at low pH, although this property differs among strains. Previous studies have disclosed several molecular mechanisms and physiological changes associated with acid resistance. However, the complete bacterial equipment used to fight against acidity is still unknown. The aim of this study was to identify all the molecular changes occurring during adaptation of *O. oeni* to acid stress. We used iTRAQ quantitative proteomics to compare the proteomes of cells exposed to mild (pH 4) or harsh (pH 3.3) wine conditions.

The proteome of *O. oeni* strain LAL01 was analyzed after exposure in a white wine adjusted to pH 3.3 or pH 4. Whole bacterial proteins were extracted from cells prepared in 3 independent replicates of each condition. After purification and iTRAQ analysis, a total of 619 different proteins were identified and quantified. Forty-four percent of these proteins were differentially expressed in the two conditions (protein quantification ratio at pH 3.3/pH 4 <0.8 or >1.2, and pvalue <0.05). Thirty-six percent (193 proteins) were up-regulated and 8 % (43 proteins) down-regulated at pH 3.3 versus pH 4. To determine their role, all the proteins were linked to the metabolic pathways of the KEGG database. Proteins up-regulated at pH 3.3 were involved in general cellular function such as DNA replication, protein synthesis or cell wall biosynthesis, but also in energetic metabolism, sugar transport, ATP production and stress resistance, including the contribution of several universal stress proteins. The results suggest that acid stress response and long term adaptation of *O. oeni* implies numerous cellular systems that modify the whole cellular metabolism and function.

## II.P.18

## ROLE OF BIOACTIVE COMPOUNDS DERIVED FROM AROMATIC AMINOACIDS ON YEAST PROTECTION AGAINST STRESSES INDUCED DURING THE ALCOHOLIC FERMENTATION

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**Keywords:** Indolic compounds, Ethanol, Antioxidant, *Saccharomyces cerevisiae*

Yeasts have to face several stresses during alcoholic fermentation, such osmotic, oxidative and acidic stress, starvation and presence of ethanol and other toxic molecules, which compromise the performance of the process. Yeasts have developed general and specific responses to increase their survival, and therefore the fermentation efficiency. These responses work in a coordinate way, so the mechanism that is triggered by one stress can induce a protective response against others. Some molecules present on wine, such as melatonin, serotonin, indolacetic acid and hydroxytyrosol, have gained attention, due to their positive effects for humans. Some of these compounds, all of them derived from the aromatic aminoacid metabolism, have been recently reported to be produced by yeast during fermentation. In a previous work, an antioxidant role of melatonin in *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts has been described, by scavenging reactive oxygen species (ROS) and by activating the antioxidant enzyme defense system. This suggests the possibility that yeast produce those molecules as protection to fermentation associated stresses. Therefore, the aim of this work was to assess if those compounds are able to protect wine yeasts against stresses related to wine industry.

Different strains of *S. cerevisiae* were grown in presence of the different bioactive compounds, and then exposed to several fermentation associated stresses, analyzing the effect of these compounds on the growth ability of stressed cells. Moreover, their physiological response was monitored analyzing different stress indicators over time: the production of ROS, the activity of protective enzymes such as catalase or the cell recovery once reinoculated into rich media. The results indicate that the effect of these compounds varied depending on the strain, the compound concentration and the stress exposition time.

For example, cells of *S. cerevisiae* grown with 5 and 50  $\mu\text{M}$  of melatonin presented better cell recovery compared with cells grown without melatonin after being exposed to 8% of ethanol during 5 hours. Lower melatonin concentrations (1 $\mu\text{M}$ ), had an effect on longer ethanol exposures (between 24–48 hours). Moreover, 5 and 50 $\mu\text{M}$  of melatonin also reduced ROS cell levels after ethanol exposition. At a glance, a protective effect of indolic compounds against some stresses, such as oxidative stress and ethanol was observed, suggesting that they could improve the fermentation efficiency.

## II.P.19

# USING CRISPR/CAS PLATFORM FOR GENETIC MODIFICATION OF COMMERCIAL *SACCHAROMYCES CEREVISIAE* STRAINS

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**Keywords:** Genomic engineering, *Saccharomyces*, Alcoholic fermentation, Interactions

The CRISPR/Cas platform, derived from a prokaryotic immune-like system, is based on an RNA guide which recognizes a specific sequence in a genome and leads a protein Cas to this sequence which proceeds to a double-stranded break. Then, cellular mechanisms will repair the DNA by Non-Homologous End-Joining or by Homology Directed Repair, which will result in genome modifications (substitution, insertion, deletion). This mutagenesis technique based on native cell repair mechanisms would allow transforming some cell strains which are difficult to modify with usual technique such as LiAc method. Commercial and indigenous strains of *Saccharomyces cerevisiae* can be genetically modified with LiAc method but with a low recombination frequency. Using CRISPR/Cas platform could allow us to increase significantly the transformation efficiency of commercial yeasts and thus knocking out these genes to understand their role or adding fluorescent genes to follow populations or gene expression.

In wine production, the understanding of cellular and molecular mechanisms set up by yeasts and bacteria in response of environmental modifications and population dynamics is essential. During alcoholic fermentation, principal actors are yeasts, especially *Saccharomyces cerevisiae* and Non-*Saccharomyces* yeasts. Transformation of these strains brings many possibilities to better understand these interactions and gene regulation. This study gives a particular interest for AMN1 gene, which takes part in flocculation mechanism, but also the HSP12 gene which is expressed during stress response. Thus beginning with these two genes of interest, this study aims to develop and optimize a transformation method in the oenological fields to allow a deeper insight in cellular mechanisms of microorganisms adaptation and interactions.

## PRACTICAL IMPLEMENTATION IN AN ENOLOGY LABORATORY OF A SPECIFIC *BRETTANOMYCES* QUANTIFICATION METHOD BY FLOW CYTOMETRY.

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**Keywords :** *Brettanomyces bruxellensis*, flow cytometry, yeast viability, specific quantification

*Brettanomyces bruxellensis* is one of the major spoilage yeasts in wine due to its capacity to produce volatile phenols and others compounds such as tetrahydropyridines responsible of irreversible organoleptic deviations.

The methods developed up to now to detect and quantify *B. bruxellensis* are problematic for different reasons: turnaround, lack of specificity, no detection of Viable But Non Culturable (VBNC) cells, enumeration of dead cells, lack of robustness. A method developed by Serpaggi et al. (2010) using fluorescence in situ hybridization (FISH) coupled to flow cytometry (FCM) using a specific fluorescent nucleotide allows specific and quick quantification of *B. bruxellensis* in red wines. The practical implementation of this method in the wine sector was developed. A two steps strategy was developed: in a first step, the presence of *B. bruxellensis* in a red wine sample, is detected by analyzing by flow cytometry, the whole cell viability by non-specific labeling of yeast. If viable yeasts are detected, a specific quantification is applied to evaluate the proportion of *Brettanomyces* among the total population of viable yeasts. This specific quantification can be used through the whole winemaking process.



# Enological Practices and Process

### III.P.1

## REDUCTION OF BAD TASTE AROMAS BY USING FILTER LAYERS WITH SELECTIVE ADSORBERS AND STUDY ON THE QUALITY OF THE WINES

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**Keywords:** TCA, FIBRAFIX TX-R filter layers, geosmin, esters

A wine defect is an unpleasant characteristic of a wine. Not repairable wine defects represent a not inconsiderable, high financial loss for wine producers. The aim of this work was to investigate, if bad taste flavors like 2,4,6-trichloroanisole (TCA), geosmin (rotten grape aroma) and ethyl phenol (so called *Brettanomyces*) can be removed by special filter layers with selective adsorbers (FIBRAFIX TX-R filter layers). Subsequently, the effect of this treatment on flavor quality of wine was examined, both sensory and analytical aspects. Using GC-SIM-MS the impact compounds as some monoterpenes and ester compounds were analyzed before and after filtration. It was found that FIBRAFIX TX-R filter layers largely reduced contents of TCA and Geosmin, but this was not applicable to *Brettanomyces* contaminated wine. In addition, it has been found that the positive flavors like esters and some monoterpenes have been greatly reduced and thus the quality has fallen significantly.

## III.P.2

## ADAPTATION TO CLIMATE CHANGE – PARTIAL DEALCOHOLIZATION OF STILL WINES TO MAINTAIN LIGHT SPARKLING WINE STYLES

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**Keywords:** partial dealcoholization, sparkling wine, sensory analysis, fermentation kinetics

Climate change give rise to growing degree days in traditional sparkling wine producing areas, which either yields still wines with higher alcohol graduation or requires very early harvesting. Due to increasing ethanol levels, onset of secondary fermentation may be severely delayed and incomplete fermentation with residual sugar and insufficient CO<sub>2</sub> pressure will occur more often. Finished sparkling wines with 13 and 13.5% ethanol lack the desired fresh and light style.

Thus we studied the potential of partial dealcoholization by osmotic distillation in a Chardonnay, Pinot Noir (Blanc de Noir) and Riesling still wine, lowering the ethanol either by 1.5 or by 3% vol. Still wines were pumped through a polyethylene based hydrophobic membrane with water at twice the velocity on the opposite permeate side. Secondary fermentation was done by the traditional method and build-up of CO<sub>2</sub>-pressure was constantly monitored by pressure-gauges. While sparkling wines of 12.5 and above % vol ethanol required more than one month for onset of secondary fermentation, which progressed slowly and often not to complete dryness, still wines with lower alcohol started much faster and finished at higher CO<sub>2</sub> levels.

After degorgement the still wines and obtained sparkling wines were analyzed by descriptive analysis, using the same odor and orally perceived attributes. In both, still wines and sparkling wines, alcohol removal stimulated lower perception of bitterness and body, while sourness was enhanced. Changes in fruity notes were not significant. Sensory differences were larger in still wines than sparkling wines, presumably due to a further increment of 1.5% alcohol and masking effects of yeast autolysis. Comparing earlier picked Riesling and Pinot Noir grapes versus later harvested riper grapes undergoing dealcoholization of their still wines, the latter variant yielded more fruity sparkling wines.

Modifications of aroma compounds in still and sparkling wines were analyzed by SPME-Headspace GC-MS, using an array of stable isotopes for quantification. Alcohol removal by osmotic distillation diminished predominantly highly volatile esters, but less monoterpenes or beta-damascenon. The degree of aroma loss was closely linked to the volatility expressed by ...

In conclusion, partial dealcoholisation seems to be a promising technology to guarantee complete secondary fermentation, allow more ripening time on the vine connected with less unripe features, and contributes to a highly appreciated sparkling wine style with less fruit and varietal expression combined with more pronounced acidity and decreased bitterness.

### III.P.3

## DIGITAL WINE FERMENTATION: PROTOTYPING OF A PRECISION CONTROL METHOD FOR THE FERMENTATION OF HIGH-VOLUME MASS-MARKET WHITE WINES

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High-volume mass-market white wines production method by means of harvest-deferred fermentation from desulphited musts is increasingly being used. Despite the advantages of this method, namely an efficient and sustainable business management, i.e., the ability to adjust the production process to the sales calendar and thus avoiding the usual seasonality in the wine sector, the aromatic profile is standardized not presenting great possibility of variation and, therefore, limiting the capacity of innovation in terms of sensory profiles. In the present work, it is envisaged the creation of a new process relying on precise management of the fermentation process to produce predetermined sensory profiles by monitoring a fingerprint of individual or groups of key-metabolites. The aromatic potential of a wild yeast, isolated under the scope of a previous research project, was tested against a commercial yeast strain. A total of 3600 hL was fermented by a controlled process using sensors to measure different key-features, while metabolic piloting was performed. Data collected throughout the fermentation process together with chemical and sensorial parameters of resulting wines, has revealed the main groups of key-metabolites, supplying an important tool to build the style of each commercial brand. The results of the ongoing work now presented, although preliminary, open good perspectives to progress from the current probabilistic situation to a deterministic production process, a clear step towards digital transformation in the wine sector.

This research was funded by Project "PRECIDIF - PRECIson Management of new Vinho Verde wines production from DIFFerential fermentation" (SI-I&DT Individuais/16/SI/2016 Projeto n° 24214). Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/QUI/00062/2019) through national funds.

## III.P.4

EFFICIACY OF GASEOUS SO<sub>2</sub> FOR BARREL SANITIZATION AGAINST *BRETTANOMYCES BRUXELLENSIS* AND EFFICIACY OF TECHNICAL ALTERNATIVES

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**Keywords:** SO<sub>2</sub> sanitization, oak barrels, *Brettanomyces bruxellensis*, alternative sanitization

Oak barrels play an integral role in winemaking because of the positive effects they impart on wine. These include enhanced aroma, colour stability and maturation. Given the expense of new oak barrels, they are repeatedly used over several years. The prolonged use of oak, however, increases the risk of microbial infection, which necessitates proper sanitation in order to prevent microbial contamination of wine.

Sulphur dioxide (SO<sub>2</sub>) is the most widely used barrel biocide used either in gaseous form or in liquid as an acidified sulphuric acid solution. Despite its popularity as barrel biocide, there is surprisingly few published scientific literature concerning the chemical and microbiological effects of gaseous SO<sub>2</sub> treatments in barrels. Therefore, this study evaluated the sulphiting of barrels by the combustion of elemental sulphur to shed some light on the efficacy of SO<sub>2</sub>.

Empty decommissioned American oak barrels (225 L) were filled with 10 L model wine having a pH of 3.4. In four barrels 5 g elemental Sulphur was combusted and they were rotated slowly. Temperatures in the barrels were measured via a thermocouple and samples of the model wine were monitored for pH and free SO<sub>2</sub> after 10 minutes, 1, 2, 5, 12, 24 and 48 hours, 4 days, 7 days and thereafter weekly for a period of 4 weeks. Within 1 h, molecular SO<sub>2</sub> reached 34 mg/L, peaking after 12 h at 43 mg/L and slowly decreased during 4 weeks to 14 mg/L; proving the microbial efficacy of gaseous SO<sub>2</sub>.

Sanitization efficacy was determined by contaminating 8 barrels with a robust *Brettanomyces bruxellensis* strain (AWRI 1499). After emptying the barrels, 4 were sanitized by combusting 5 g/L sulphur and 4 were kept untreated as controls. Microbial sampling took place after 1, 12 and 24 h and 1, 2, 3 and 4 weeks: From each barrel 3 oak discs were removed by means of a circular saw and the holes were immediately closed by silicon plugs. *Brettanomyces* cells were extracted from the oak discs and living versus dead cells was determined by flow cytometry. While untreated barrels showed 3 x 10<sup>6</sup> cells, sanitization by gaseous SO<sub>2</sub> yielded a more than 2.5 log reduction after one week, proving the efficacy of microbial sanitization.

Alternatives such as treatment with hot water, steam, ethanol, ozone, sodium-per-carbonate or per-acetic acid were tested in a model system using contaminated oak staves. The highest log reduction of 3.5 for *Brettanomyces bruxellensis* cells (strain AWRI 1499) was achieved by ozone.

### III.P.5

## EXTRACTION OF PROANTHOCYANIDINS FROM SKINS AND SEEDS OF CABERNET SAUVIGNON IN WINE-LIKE SOLUTION

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**Keywords:** proanthocyanidins, extraction, grape seeds, skins

Extraction of proanthocyanidins (PAs) from skins and seeds in wine-like solution was studied. The content and structural characteristics of extractable PAs were determined spectrophotometrically and by UHPLC-DAD-MS/MS, respectively. Skin and seed extracts were characterized both chemical and sensorial from 3 till 20 days of extraction. High molecular weight PAs (HMWP) extraction from seeds increased continuously till 20 days whereas low molecular weight PAs (LMWP) reached plateau after 7 days.

The extraction of both HMWP and LMWP from skins was the highest already at first sampling, after 3 days. Sensory evaluation confirmed higher astringency and bitterness of seed extracts with increasing time. There were no differences in astringency and bitterness of skin extracts between extraction days.

Regarding structural characteristics, seed and skin extracts didn't statistically differ in mean degree of polymerisation (mDP) and in percent of galloylation (%G) between extraction days (with exception of seeds at first sampling).

During extended extraction of seeds in wine-like medium the continuous growth of HMWP content was more evident than structural changes in mDP and %G.

**III.P.6****INFLUENCE OF BOTTLE POSITION UPON CORK STOPPER RESILIENCY AND SPARKLING WINE COLOUR. A FOUR YEARS' STUDY AFTER CORKING****Richard MARCHAL**

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**Keywords:** Cork resiliency, storage bottle position, sparkling wine color, Champagne aging

After Champagne popping, the first consumer's observation is the shape of the cork stopper. Consumers expect a "mushroom shape". Nevertheless, we sometimes observe a "barrel" shape due to inappropriate cork's elastic properties. The aim of this study was to follow the loss of cork stopper resiliency during four years, according to the bottle position and cork quality. Two geographical origins (corks A and B) were compared. For each origin, a high and a medium qualities obtained by visual selection were studied. A total of 720 bottles of sparkling wine were closed and stored vertically (Vb) or horizontally (Hb). Bottles were open after 1 to 4 years to follow cork resiliencies and wines' colour (4th year). During the 4 years of the study, significant differences (ANOVA) were observed between the resiliencies of Vb-cork and those of Hb-cork, whatever the cork origin and its quality. The Vb-cork diameters were systematically higher and moreover the differences in resiliency increased with time. The heterogeneity of the cork diameters was also strongly higher among Hb-cork than for Vb,-cork, evidencing the importance of the bottle storage position after corking. Concerning Hb-cork, a highly significant difference was observed between corks from region A and corks from region B whatever the quality.

Four years after corking, the colour of the white wines was measured using the CIE-L\*a\*b\* method and the absorbance at 420 nm (A420). These data were compared by ANOVA. Whatever the geographical origin of the cork (A or B) and its quality (high or medium), significant differences were observed between wines from bottles stored horizontally and wines from bottles stored vertically for the a\*, b\* and A420 parameters. When bottles were stored vertically, all of the wines presented higher b\* (more yellow) and A420 values, corresponding to a higher micro-oxygenation and leading to more mature wines. These observations are in agreement with the level of resiliency of the cork. A higher cork elasticity facilitates more oxygen micro-ingress leading to a slightly quicker wine maturation.

As a conclusion, this 4-years study clearly showed that the position of the sparkling wine bottle during the storage after corking is able to influence the visual aspect of the cork as well as the colour of the wine. This kind of storage (vertical/horizontal) could be used as a tool to manage wine maturation after corking.

### III.P.7

## MACERATION STEP EFFECT ON PHENOLIC COMPOSITION AND BIOLOGICAL ACTIVITIES OF CABERNET SAUVIGNON AND SYRAH MUSTS IN LEBANON

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**Keywords:** Maceration , Phenolic compounds , Biological activities , Cabernet Sauvignon

The attribution of beneficial health effects to the consumption of wine goes back to the highest antiquity. These beneficial effects are attributed to the presence of phenolic compounds especially in red wines.

The extraction of these compounds from the grape takes place mainly during the maceration phase. The conduct of the maceration depends mainly on the winemaker choices and should be regulated to favor the dissolution of the phenolic compounds to the maximum. The aim of the study is the determination of the impact of maceration step (pre-fermentation /cold, hot, with or without added enzymes) and the maceration time on the extraction kinetics of phenolic compounds and on biological activities of Cabernet Sauvignon and Syrah musts.

Red grapes of *Vitis vinifera* var. Cabernet Sauvignon (CS) and Syrah (Sy) were supplied by two Lebanese cellars: Clos St. Thomas and Chateau Florentine. Pre-fermentative macerations were conducted at different temperatures (10, 60, 70 and 80 °C) for 0, 2, 4, 8, 24 and 48 hours by analyzing the phenolic composition by spectrophotometric and chromatographic methods. Then, the biological activities (antioxidant (ABTS and DPPH), anti-lipoxygenase (anti-inflammatory), anti-cholinesterase (anti-Alzheimer), anti-xanthine oxidase, anti- $\alpha$ -glucosidase (antidiabetic) and cytotoxicity activities (anticancer) were realized.

The results presented in this study highlight that the phenolic composition of musts is greatly affected by the maceration step. The pre-fermentation heat treatment of grapes is more efficient for the extraction of polyphenols than the cold maceration. Analysis of must samples revealed a systematic increase in the concentration of tannins with temperature and over time. Temperature favored anthocyanin extraction but a degradation was observed at high temperatures when the maceration is extended beyond 8 hours. Results showed also that temperatures of 10°C and 60°C showed remarkably low biological activities for the different musts compared to 70°C and 80°C from Syrah Saint-Thomas. The same low activities were noticed for Cabernet Sauvignon Florentine macerated at 80°C after 48 hours. Syrah Saint-Thomas macerated at 70°C exhibited the highest inhibition percentage for most of the biological activities studied. Biological activities analyses of musts showed that higher antidiabetic and anti-inflammatory activities were more correlated to the high anthocyanin and phenolic acid content.

## III.P.8

## NEW TOOL FOR MICROBIAL STABILIZATION: STUDY OF THE IMPACT OF MICROWAVES ON OENOLOGICAL MICROORGANISMS

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**Keywords:** Microwaves, Microbial stabilization, *Brettanomyces bruxellensis*, wine

Nowadays several procedures have been put in place to limit and stabilize microbial communities during wine production. These processes can be divided into two categories: chemical and physical methods. Chemical methods for limiting microbial communities consist of the use of inputs during winemaking with sulfur dioxide (SO<sub>2</sub>). In addition to the toxicity of SO<sub>2</sub>, the growing consumer demand for products with as few inputs as possible leads winegrowers to turn to other methods to limit wine deterioration due to microorganisms. Thus, the development of emerging technologies for physical wine stabilization as UVc treatment, pulsed electric field or microwaves in order to reduce the utilization of SO<sub>2</sub> could be essential to winemakers.

In this study we investigated microwaves (MW) stabilization effect on a red wine naturally contaminated and a wine model solution (grape juice 250ml/L; 5g/L yeast extract; Tween80 1ml/L; pH 3.5; 12% ethanol) contaminated with lactic acid bacteria and commercial yeast. The microwaves treatment was tested at a power of 150W at 50, 60 and 70°C during 20s. The microorganism enumeration results show in the naturally contaminated wine that 50°C doesn't have a significant impact on total yeast, *Brettanomyces bruxellensis* and on lactic acid bacteria. However, at 60°C of MW treatment only total yeast are still present with 2 log of reduction. In the wine model solution, we observe the same trend, with no significant effect of MW at 50°C but at 60°C all the *Oenococcus oeni* population is destroyed and 2 log of reduction for total yeast. At 70°C, none of each microorganism are still alive in wine and in the model solution. An observation of cell with scanning electronic microscopic at different MW treatment show multiple effect on wall cell increasing according to the temperature.

According to these preliminary results, microwaves seem to have a lethal effect on wine microorganisms since 60°C and need further investigation on sensorial impact.

### III.P.9

## SKIN POLYPHENOL EXTRACTION DURING MACERATION: A QUITE COMPLEX PROBLEM?

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**Keywords:** grape skin polyphenols , extraction coefficients, diffusion, model solution

According to literature, the extraction of skin polyphenols in winemaking is mostly related to their solubility and to their interactions with cell wall material, which forms a barrier against their diffusion. In the present work, the impact of the grape variety and maturity on the extraction of skin polyphenols was studied in model systems.

Two grape varieties with contrasted polyphenol compositions (Carignan, Grenache) were sorted according to their degree of maturity and their size. Berries were manually peeled and skins recovered for biochemical analyses and extraction experiments. Analyses included polyphenol constituents, insoluble cell material (ICM) polysaccharides and proteins in ICM. Extraction experiments were performed on fresh skins, by increasing ethanol percentages from 0 to 15% to mimic maceration during fermentation. Polyphenol concentration was measured by UV-Vis spectrophotometry, HPLC and SEC. After extraction in wine-like conditions, skins were transferred several times in fresh solvents (0 then 15% ethanol), until no further diffusion was observed. Extractable polyphenols in wine-like solvents were determined from these experiments and compared to (i) polyphenols extracted at the end of the maceration and ii) polyphenols extracted with a good solvent (acetone/methanol/water) on both fresh and extracted skins.

Extraction profiles during maceration strongly differed between the two varieties and with the berry ripeness. Analyses indicated that these differences were first linked to the initial polyphenol composition in skins. In fact, polyphenol extraction coefficients at the end of the maceration (amount extracted/amount in skins) depended on their amount in skins but also on the anthocyanin to tannin ratios (A/T). Maximum 50 % of skin phenolics were extracted in Grenache berries in comparison to 30% in Carignan. However, other factors than the phenolic composition could participate in these differences, such as ICM composition. No significant differences between the different varieties and modalities were observed, but structural differences cannot be ruled out.

Finally, after successive washings with fresh solvents, up to 90-92% of skin polyphenols were extracted in wine like conditions. This showed that interactions between skin insoluble and polyphenols modulate their extraction but do not have a major impact on the total amount of extractable skin polyphenols.

## III.P.10

**ENZYMES PLUS ULTRASOUNDS AS TOOLS FOR INCREASING GRAPE PHENOLIC EXTRACTION****Encarna GOMEZ-PLAZA**

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**Keywords:** enzymes, ultrasounds, tannins, phenolic compounds

In this study we propose the combined use of maceration enzymes and high power US for increasing the phenolic extraction from grapes and/or limiting phenolic adsorption of these phenolic compounds in suspended solids during a red wine vinification. For this, six different types of microvinifications have been made on laboratory scale, in which ultrasound and maceration enzymes have been used separately and together at two different moments of skin maceration. Since both treatments affect the structure of the cell wall and aim to increase and accelerate the extraction of phenolic compounds from inside skin cells of grape, we want to test if they could have a synergistic effect. Moreover, the US have been commonly applied after the crushing of grapes and before the beginning of the maceration, forgetting that some phenolic compounds such as proanthocyanidins (both skin and seed) may improve their extraction in the presence of ethanol in the medium, so the effect of the US may be increased if it is present. The chromatic characteristics of the different microvinifications were analyzed by spectrophotometry and the concentration and composition of proanthocyanidins by high resolution liquid chromatography and size exclusion chromatography. All the analyzes were made at the end of alcoholic fermentation. Large differences were observed depending on the moment of high power ultrasound application, the best results observed when the maceration enzymes were applied just after crushing the grapes and the ultrasound applied after three days of skin maceration. Our conclusion is that the skin structures are damaged after enzyme action and three days of skin maceration and at that moment, the application of ultrasounds led to a very important destruction of the already damaged skin structures, increasing the tannin extraction up to 42%.

### III.P.11

## IMPACT OF GLUTATHIONE-RICH INACTIVATED YEAST ON WINE STABILITY

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**Keywords:** yeast derivative, radical scavenging, nucleophilic index, aging potential

Glutathione-rich inactivated dry yeasts (GSH-IDYs) are claimed to accumulate intracellularly and then release glutathione in the must. Glutathione is beneficial to the wine quality, but scientists also highlighted that GSH-IDYs have a greater effect than only increase the pool of this antioxidant in the wine. This work aimed at estimating quantitatively and qualitatively the ability of yeast derivatives to act as stabilization agents towards oxidative processes occurring in wine.

An electrophilic derivatization strategy was applied to estimate the pool of nucleophilic compounds present in the model wine containing the soluble fraction of IDYs with increasing intracellular GSH content. This allowed to estimate (in glutathione equivalent) the fraction which reacted with the electrophilic probe by High-Resolution Liquid Chromatography Mass Spectrometry (HR-LC-MS). Simultaneously, the DPPH assay specifically developed for wine like medium was used to estimate the total scavenging activity of the IDYs.

The DPPH assay enabled to have a first overview of radical scavenging capacity of IDY soluble fractions. In this experiment, the most efficient IDY was 9 times more active than the weakest to scavenge the DPPH radical. No direct correlation could be observed between actual concentrations of glutathione in the solution and DPPH scores. This revealed the likely implication of other compounds involved in the radical stabilization reaction. The aim of the derivatization is to catch sensitive nucleophilic functions such as sulfhydryl (aromatic thiol or peptides containing cysteines) and improve their detection. The eight IDYs showed different diversity and concentration of 10 dominant nucleophiles detected in positive ionization mode by HR LC-MS. A new index was created based on the diversity, the abundance and the affinity of each nucleophile for this specific electrophile probe. This index allowed to classify IDYs according to a nucleophilic power. Comparison to the DPPH scores revealed similar trends but also showing more complex relationships between the composition and the activity of such soluble fractions.

This new approach consisting in the determination of the nucleophilic power of a sample is a promising tool not only for yeast derivatives used as wine stabilizer, but also in winemaking to estimate the aging potential of a wine before bottling.

## III.P.12

**APPLICATION OF GRAPE CRUSHING TO INCREASE PHENOLIC COMPOUND EXTRACTION DURING WINEMAKING****Sergio GÓMEZ-ALONSO**José Pérez-Navarro<sup>1</sup>, Adela Mena-Morales<sup>2</sup>, Esteban García-Romero<sup>2</sup>, Pedro M. Izquierdo-Cañas<sup>2,3</sup>

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**Keywords:** phenolic compounds, extraction, crushing, wine

Phenolic compounds constitute a major class of secondary metabolites found in plant tissues. The study of these compounds in wine is of great interest since they are related to wine sensorial properties, such as color, astringency, and aging aptitude. A high proportion of phenolic compounds remains in grape tissues after the fermentation/maceration process, being this a varietal grape characteristic. Temperature and length of maceration had showed significant impacts on the rate of extraction of phenolic compounds to wine, moreover different oenological practices have been developed to improve the effectiveness of the extraction in the last years.

The effects of grape crushing during the elaboration of Monastrell red wines have been evaluated in this work. The experiment was carried out following three procedures: crushing the grape skins before (P) and during (D) fermentative/maceration stage, and an elaboration without crushing (C). Wine phenolic composition was analyzing using HPLC-DAD-ESI-MS/MS.

Application of grape crushing before pre-fermentative/maceration (P) caused a significant decrease of the non-acylated anthocyanins, pyranoanthocyanins, hydroxycinnamic acid derivatives and stilbenes. However, both crushing treatments had no effect on the total concentration of flavonols. A significant increase of flavan-3-ol monomers, dimers and polymers content were determined in all crushed wines, showing also treated wines higher galloylation percentage and lower proportion of prodelphinidins. Data obtained suggest that both crushing treatments increase the flavan-3-ol extraction during winemaking and may affect the sensory profile of wines.

**III.P.13**

**CHARACTERIZATION OF OAK BARREL SPECIFIC OXYGEN PERMEABILITY**

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**Keywords:** barrel, permeability, transfer, oxygen

Aging in the barrel is an important and traditional practice during red wine-making in Bordeaux. In addition to the dissolution of aromatic wood compounds, the extraction of wood tannins in the wine and the amelioration of colloidal stability, it is commonly accepted that the natural micro-oxygenation process during barrel aging participate in the global evolution of the chemical composition of wine. Thus, many studies have been performed on the oxygen transfer through barrels by researchers [1].

Most of the time, the gas exchange during aging is evaluated by measuring the increase in dissolved oxygen or by measuring gas transfers through specific parts of the barrel. However, these methods do not take into account the whole barrel or the consumption of oxygen by wine compounds, thus the results may be inaccurate. This study aimed at measuring oxygen transfer of empty barrels thanks to micro-GC (gas chromatography coupled with a plasma emission detector) and thanks to a stainless steel hermetic tank containing the barrel. We propose to show the first results concerning the impact of humidity of oak wood on the oxygen transfer rate of the barrel.

[1] del Alamo-Sanza, M.; Nevares, I.; *Crit. Rev. Food. Sci. Nutr.*, 2017, 1-16.

## III.P.14

DOES THE TEMPERATURE OF THE PRISE DE MOUSSE AFFECT THE LOSSES OF DISSOLVED CO<sub>2</sub> AND THE COLLAR OF CHAMPAGNE AND SPARKLING WINES?

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**Keywords:** Sparkling wines, CO<sub>2</sub>, Collar, Temperature

Champagne and sparkling wines elaborated through the traditional method are supersaturated with CO<sub>2</sub> forced to dissolve progressively in the liquid phase during a second in-bottle alcoholic fermentation (the so-called prise de mousse). The prise de mousse is followed by a minimum aging period of 15 months in contact with lees. Actually, a standard 75 cL champagne bottle typically holds about 9 g of dissolved CO<sub>2</sub>.

This content of dissolved CO<sub>2</sub> in the liquid phase is responsible for bubble formation once the bottle is uncorked and the wine poured into a glass. Moreover, dissolved CO<sub>2</sub> is a parameter of high importance since it directly impacts the following sensory properties: the frequency of bubble formation, the growth rate of rising bubble, the very characteristic tingling sensation in mouth, and the overall olfactory perception of champagne. Champagne and sparkling wines also contain many compounds originating from grape and yeast that might influence the stability and the height of the collar in the glass. Moreover, during aging on lees, the dead yeasts undergo autolysis, and progressively release different compounds in the liquid phase that progressively modify the wine composition and most likely its organoleptic properties (such as its aromas, and foaming properties).

Traditionally, both the prise de mousse and aging on lees are undergone in cool cellars, at a temperature close to 12°C. Higher temperatures might likely influence the release of compounds by yeasts, as well as the chemical and biochemical reactions undergone by the wine. As such, this study was aimed at unveiling the impact of two distinct temperatures for the prise de mousse (namely, 13 and 20 °C) on several characteristics of sparkling wines in real tasting conditions: (i) the progressive losses of dissolved CO<sub>2</sub> as well as (ii) the collar height and (iii) the average bubbles' size in the foam collar. Three batches of sparkling wines were chosen: two Champagne wines and one Crémant de Loire. A campaign of analysis was carried out on wines that were disgorged two weeks after the end of the secondary fermentation. Our results showed that the sparkling wines elaborated at 13°C and served in standard tasting conditions (i.e., 100 mL, 12°C) had a better ability to keep dissolved CO<sub>2</sub> in the liquid phase than those elaborated at 20 °C. Most interestingly, we also observed that the wines which underwent their prise de mousse at 13°C presented slightly thinner bubbles in the foam collar.

### III.P.15

## EFFECT OF A GLUTATHIONE ADDITION ON WHITE MUST DURING PRE-FERMENTATION STAGE

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**Keywords:** must, glutathione, oxidation, thiols

Effect of glutathione addition on white must has been tested on Melon B. and Sauvignon B. variety produced in Loire Valley vineyards. The experimental system aimed to compare, on must during juice extraction, the addition of glutathione with non treated juice and usual sulfites addition (50mg/L).

Glutathione amounts varied from 50 to 200 mg/L. A mixed sulfites (25 mg/L) - glutathione (50 to 100 mg/L) was also tested. The must thus obtained were subsequently processed as usual in order to make traditional white wine. The main factors selectionned to follow up the trial were must colour, thiols concentration and sensory profiles of the wines. In the light of knowledge on enzymatic browning, results had demonstrated that glutathione addition could protect must from oxydation to some extent.

The observed effect is similar to a 50 mg/L sulfite addition, which remains the reference. In cases where glutathione addition were larger, a substantial amount of glutathione is likely to stay available in the must at the moment of the alcoholic fermentation (nitrogen source for yeast ? protection of the wine from oxydation ? sulfur compounds leading to reduction taste ?). On the aromatic composition terms, wines made with glutathione boosted musts have the same thiols concentration than wines made with musts protected with sulfites, and higher thiols concentration than the non protected one. Elsewhere, the mixed addition of sulfites and glutathione to protect the musts has been the modality wich gives the wines with the highest content of thiols. Considering sensory profiles, wines thus obtained are judged similar to traditional wines and better than wines made without protection before fermentation. In case of massive glutathione addition, the aromatic defaults associated with volatile sulfur compounds increase. At the end, glutathione use on must could be an alternative to sulfite addition to fight against oxydation. Nevertheless, it remains a question of the appropriate dose, which needs to be calibrated according to the inherent sensibility of the must and oxygen exposition caused by the grapes treatment process.

## III.P.16

## EFFECT OF SHAPE AND MATERIAL OF THE TANKS USED DURING ALCOHOLIC FERMENTATION OVER CHEMICAL COMPOSITION AND SENSORY ATTRIBUTES OF SAUVIGNON BLANC WINES.

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**Keywords:** sauvignon blanc, tank material, alcoholic fermentation, chemical composition

Nowadays a great diversity of tanks for winemaking are available in the market. Wineries still use traditional tanks such as stainless steel deposits or oak barrels, but the use of novel tanks made from other materials (such as concrete, polyethylene or clay) and with different shapes (such as the fashionable oval shaped tanks) is spreading. However, little is known about the real impact of using these novel tanks on the final properties of wine.

By this work we pretend to assess the impact of shape and material of tanks used during wine alcoholic fermentation on the cellar over the final qualities of Chilean Sauvignon Blanc wines. For this purpose a batch of Sauvignon grape juice (22.1 brix, pH: 3.4, titratable acidity 4.41 g/L of tartaric acid equivalents) from the 2018 harvest (Leyda Valley, Chile) were fermented by using four kinds of tanks by triplicate: (1) stainless steel tanks, (2) clay jars, (3) polyethylene oval tanks and (4) concrete oval tanks. When de alcoholic fermentation was completed the wines were bottled and stored in a dark cellar until analysis. Physical, chemical and sensory analyses were performed.

The alcoholic fermentation delays 16 days for stainless steel tanks, while it delays 11 days for all the other tanks. At the end of alcoholic fermentation, stainless steel tanks and clay jars shows the lower residual sugar and the higher volatile acidity. The concrete oval tanks shows statistically higher conductivity, pH, and total phenols (when analyzed by spectrophotometric measurements at 280 nm). Some slight differences were observed on wine color among tanks; being the wines fermented on clay jars those had the higher color intensity. Focusing on elemental analysis (by ICP-AES) the wines from concrete oval tanks shows higher amounts of potassium, phosphorous, magnesium and manganese. No statistical differences among wines were observed on phenolic composition when the low molecular mass phenols were analyzed by HPLC-DAD. Focusing on volatile compounds (SPME-GC-MS) the polyethylene oval tanks shows the lower total concentration, but the effect of tank is different for each family of volatile compounds. Regarding sensory analysis the concrete oval tanks shows statistically lower bitterness and fruity scents.

*Acknowledgements* The authors would to thank CONICYT-Chile for financial support (Project FONDECYT 11160510).

### III.P.17

## EFFECTS ON VOLATILE PROFILE BENTONITE ADDITION BEFORE AND AFTER TIRAGE TO PRODUCE TRADITIONAL SPARKLING WINES

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**Keywords:** Sparkling wine, Volatile compounds, Fining agent, Bentonite

During sparkling wine production, bentonite is widespread used as fining agent to achieve the protein stabilization of the base wine, but also as agent to facilitate the riddling process. The lack of an industrial-scale available alternative for bentonite use during sparkling wine production explains its widespread employment in wineries, despite its negative effect over foam quality and aromatic profile. This is due because bentonite is not selective and removes other molecules besides unstable proteins, which can decrease the final quality of wine. However, a certain amount of bentonite is required to reach the protein stabilization of the base wines. Thus, this bentonite dosage could be distributed among both: the dosage during fining of base wines and the dosage in the tirage liquor as riddling adjuvant.

Therefore, a study of the impact of the distribution of the required amount of bentonite between the fining of the base wine and the addition in the tirage liquor (by using different proportions for each process) was carried out, in order to know the best conditions to reach the protein wine stability minimizing the negative influence of the treatment on foam and aromatic potential.

For this purpose, 60 liters of Chardonnay base wine were divided in three batches of 20 liters in stainless steel tanks. The bentonite dosage needed for the protein stabilization determined was 17 g/hL. The 50% of the dosage was added to the first tank, the 75% to the second tank and the full dosage to the third tank. After 1 week the bentonite was retired. During the tirage, the bottles from the first tank were spiked with the remaining 50% of the dosage, the bottles produced from the second tank with the 25% and finally no bentonite was added to the bottles from the third tank.

General analytical parameters were analyzed following the official methods of OIV and volatile compounds were extracted by Headspace Solid Phase Micro-extraction (HS-SPME) and analyzed by gas chromatography coupled to a mass spectrometer (GC-MS).

The samples analyzed were the base wine before addition of bentonite, after the first addition of bentonite, after second fermentation (second dosage of bentonite added) and after 9 months of aging on lees.

The results showed that, to produce sparkling wines with high amount of volatile compounds the best choice was to apply 100% of the bentonite dosage before the tirage because, in spite of the lost of volatiles before the tirage, during the second fermentation the absence of bentonite allowed mainly to preserve the esters compounds produced in the wine. Also terpenes and alcohols (mainly 2-phenylethanol) were present in significant higher quantities in these wines. After 9 months of aging period, these differences were maintained and even became more relevant.

*Acknowledgements: Authors want to thank CONICYT (FONDECYT 1171227) for the financial support.*

## III.P.18

## EVALUATING THE EFFECTIVENESS OF FIVE COMMONLY USED STRATEGIES FOR THE REMEDIATION OF 'REDUCTIVE' AROMAS

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**Keywords:** Hydrogen sulfide, Remediation, Copper, Oxygen

Volatile sulfur compounds (VSCs) are known to impart 'reductive' aromas in wine, described as 'rotten egg' (hydrogen sulfide, H<sub>2</sub>S), 'putrefaction' (methanethiol, MeSH) and 'rubber' (ethanethiol, EtSH). These compounds play important roles in determining wine aroma, consumer preference, and the perception of wine quality.

Winemakers make use of various remediation strategies to manage 'reductive' aroma formation. Traditionally, copper fining is one of the most commonly used remediation strategies, and it is known to be effective in the short term (i.e. weeks to months). However, during long-term storage (i.e. months to years) excess copper remaining in the wine post-treatment may increase the risk of liberating VSCs in wines post-bottling. Other remediation techniques such as DAP additions, oxidative handling and racking, and fresh lees addition are also commonly employed to treat unwanted VSCs.

In this study, the relative effectiveness of five commonly used 'reductive' aroma remediation strategies were compared. Five wines (in triplicate) were prepared and on the onset of 'reductive' aromas, each wine was treated with a unique remediation strategy, namely: (1) copper fining, (2) macro-oxygenation, (3) a combination of macro-oxygenation and copper fining, (4) diammonium phosphate (DAP) and (5) the addition of fresh lees.

The effectiveness of the different treatments was evaluated over 12 months using chemical and sensory analysis techniques. All the remediation techniques had varying levels of effectiveness in treating the 'reductive' aromas. Overall, the combination of macro-oxygenation and copper fining appeared to be the most effective in giving lowest 'reduction'-related attributes while enhancing 'fruity' attributes, while copper fining alone, lees, and to a lesser extent DAP addition, were shown to diminish fruit attributes and confer 'reductive' characters.

III.P.19

**EVOLUTION OF PHENOLIC AND SENSORIAL CHARACTERISTICS OF ROSÉ WINES AGED IN CONTACT WITH ALTERNATIVE WOOD CHIPS FROM OAK AND CHERRY**

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**Keywords:** aging, cherry, oak, rosé wine

According to OIV (2015), in 2014, global production of rosé wines concerns 9.6 % of the world still wine production. Thus, the rosé wines represents a growing trend in the wine market. As a result of the specific winemaking process used during rosé wines production, these wines had significantly lower phenolic compounds levels than red wines. However, there are several possibilities to change the phenolic content of rosé wines and consequently contribute to produce rosé wines with different phenolic profile and sensory characteristics. Thus, one of the potential possibilities to introduce changes on rosé wines characteristics is to use wood chips during the aging process.

Actually, only wood from *Quercus* (oak wood) and *Castanea Sativa* (chestnut wood) species are recommended by OIV and authorized by European Union for oenological use. However, in particular for oak wood, there is an increase demand of these wood, which caused a remarkable potential increase in costs due to the limited availability of materials and also an ecological impact of cutting the oak trees in forest. Thus, the use of other wood species, such as, cherry (*Prunus avium*), may be an interesting option for the wine production. In this context, in order to deepen the knowledge of the potential impact of the use of wood chips from oak and cherry on rosé wines phenolic composition, the present study aimed to evaluate the color, pigments and various phenolic parameters, and also individual phenolic compounds by HPLC-DAD (anthocyanins and some molecular weight flavanols) of rosé wines produced in contact with oak and cherry wood chips (1.5 g/L). In addition, a sensorial evaluation was also conducted to analyze the potential sensory changes of rosé wines during the aging process. This experimental work was made in an industrial scale in a winery using grapes from Touriga Nacional grape variety.

Concerning to the results obtained, both woods improved wine color intensity and pigments stability, which was significantly more relevant when was used cherry wood chips. In addition, total phenolic content was consistently higher in rosé wines also aged with cherry wood, in comparison with the control and oak wines, the latter even showing less total phenols than the control wine. Both wood rosé wines also had a considerably larger proportion of colored anthocyanins than the control wine. Rosé wines aged in contact with cherry wood chips also showed a much higher concentration of flavanols. Finally, from the sensory analysis, the wines elaborated with wood chips always were scored highest in overall rating (cherry at 40 and 60 storage days, oak wood at 80 days), significantly improving color intensity and overall quality, as well as several aroma attributes.

## III.P.20

## IMPACT OF STALK CONTENT ON WINE CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS DURING GAMARET SWISS WINE VINIFICATION

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**Keywords:** grape stalk, tannins, whole grapes, gamaret

Using a proportion of whole grapes is a traditional red winemaking technique used around the world that comes back to fashion. Studies have shown that stalks can bring many benefits to the wine such as alcoholic reduction, color protection or improvement of the tannic intensity. The study aims to identify the relevance of using this type of winemaking in the case of wines made from Swiss grape varieties, such as Gamaret. Gamaret grapes from a vineyard in Gland (Switzerland) were harvest and processed differently. Two trials were performed without stalks, they were used as control. Different modalities of stalks addition were tested including 10% and 20% of whole grapes added to the rest of the destemmed harvest. Each trial was carried out on a harvest volume of 150 kg. Different analytical methods were used in order to identify the influence of the stalks on the wine organoleptic properties. Classical parameters (alcohol, acidity, pH, SO<sub>2</sub>, ...) were determined by FTIR and colorimetric methods. Polyphenol content was evaluated using spectrophotometer measurements (IPT and total tannin content) and oxidizable compound content was quantified using the NomaSense PolyScan P200 (Vinventions). Chemical analysis of the wines vinified with stalks showed a decrease in alcohol content, lower pH values and less color degradation. These results are consistent with the available previous studies on stalk winemaking. Interesting results showed the importance of the stalk proportion on the extractability of oxidizable compounds. The sensory analysis of these wines was performed and the use of stalks during winemaking appeared to be benefic in terms of mouth structure and tannin quality.

### III.P.21

## IMPACT OF TEMPERATURE AND HUMIDITY CONDITIONS IN THE CANTEIRO AGEING OF MADEIRA WINES: PRELIMINARY RESULTS

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**Keywords:** wine science, fortified wine, accelerated ageing, real-time monitoring

Madeira wine is a well-known fortified wine (17–22% ABV) from the island of Madeira, Portugal. The peculiar winemaking process, in particular the ageing step, makes this wine unique in the world. During the canteiro ageing, Madeira is aged in oak casks for at least 3 years at temperatures higher than usual in still wines, wherein the casks are specifically placed in naturally heated winery lofts.

The exposure to these conditions has a great impact on the development of the unique features and overall quality of Madeira wine [1].

Considering the impact of this wine in the regional economy and on its international projection, a project is currently ongoing in order to know the impact of temperature and humidity on its ageing evolution. To do so, Madeira wine warehouses were real-time monitored and two batches of 2018 Tinta Negra wine (one dry and other sweet) were distributed in four 225 L oak casks between the warehouses located in different places. The wines were analysed in terms of oenological parameters and individual organic acids (HPLC-DAD).

Until now, great differences were found in temperature (about 2 °C) and humidity (about 20%) which is expected to produce differences in the analytical data and on the wines organoleptic characteristics.

#### Acknowledgements

Vanda Pereira is thankful to ARDITI (Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação) for funding her research grant under the project M1420-09-5369-FSE-000001. This work was financed by ERDF funds and National Funds through the FCT (Fundação para a Ciência e Tecnologia) under the projects UID/CTM/50025/2013 and M1420-01-0247-FEDER-000020 (IMPACT III).

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## III.P.22

## IMPACT OF THE PRE-FERMENTATIVE ADDITION OF ENOLOGICAL ADJUVANTS ON THE DEVELOPMENT OF UTA IN WINES

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**Keywords:** untypical ageing defect, 2-aminoacetophenone, adjuvants, high-resolution mass spectrometer

The presence of reasonable amount of 2-aminoacetophenone (AAP) in wines is regarded as the main cause of untypical ageing defect (UTA) described by aroma descriptors such as "acacia blossom", "furniture polish", "wet wool", "mothball", or "fusel alcohol" [1, 2]. Indole-3-acetic acid (IAA), the main naturally occurring auxin present in plants, was identified as the principal precursor of this molecule. In plant, a significant part of IAA is usual conjugated to amino acids by IAA-amido synthetases [3] or stored as ester conjugates of sugar moieties [4], becoming a reserve of IAA in must. Nevertheless, the final amount of free IAA in wines is also influenced by the metabolism of microorganisms involved in winemaking process, in particular yeasts which absorb and metabolize amino acids. Tryptophan is oxidatively deaminated, via indole-3-pyruvic acid (IPA) to indole-3-lactic acid or, via IPA and indole-3-acetaldehyde, reduced to tryptophol or oxidized to IAA [5].

The present study aimed to evaluate the effectiveness of different enological adjuvants (grape tannin, GrT; ellagic tannin, EgT; gallotannin, GaT; ascorbic acid, ASC; glutathione, GSH) added to musts in pre-fermentation for preventing the development of UTA during a forced aging of wines (6 days at 40 °C, dark). Johannitter, Pinot Blank, Pinot Gris and Riesling musts were separately added of the 5 adjuvants (250 mg/L GrT, EgT and GaT; 100 mg/L of ASC; 20 mg/L of GSH), fermented and finally added of sulfur dioxide (50 mg/L). The wines were then analysed to quantify AAP and its precursors, before and after the aging treatment. The quantification was performed using a high performance liquid chromatograph equipped with a pre-concentration and purification SPE-online system and coupled with a high-resolution mass spectrometer (UHPLC-HQOMS) using a biphenyl column (3x150 mm, 2.7 µm) with formic acid 2% and acetonitrile as eluents. The quantification limits ranged from 0.25 to 2 µg/L, excepted for AAP that had a quantification limit of 0.02 µg/L.

The heating induced in wines the formation of APP up to concentration of 4 µg/L. The best protection against AAP formation was achieved using ASC (AAP always <0.6 µg/L) and GaT (<1 µg/L).

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### III.P.23

## INFLUENCE OF GRAPE SKIN CELL WALL MATERIAL ADDITION ON THE POLYPHENOLIC COMPOSITION AND SENSORY PROPERTIES OF RED WINES

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**Keywords:** astringency, color, grape cell wall material, phenolic composition

Phenolic maturity and sugar maturity are two important indexes for enologists since they will further influence wine quality. In the past decades, the delay between these two kind of maturity has increased because of global climate change, which lead to wines with poor quality (too astringent and poor color) due to the inadequate phenolic composition of the grapes at harvest time [1]. For that reason, several strategies have been proposed to solve this problem and, among them, the addition of polysaccharides.

Polysaccharides from grape skin cell wall material (CWM) are able to interact with polyphenols (mainly anthocyanins and tannins, the primary responsible of wine color and astringency) through hydrophobic and hydrogen bonds [3]. The interaction effectiveness depends upon the composition, concentration, molecular size and structural flexibility of both polyphenols and polysaccharides from CWM [3].

It has also been reported that polysaccharides from CWM could affect protein/tannin interactions, which could influence wine astringency [2]. Moreover, tannins in wines not only determine wines' astringency, but also play an important role in stabilizing wine color because of their interaction with anthocyanins.

In the present study, the effect of the addition of CWM on the phenolic composition and color of a red wine, showing an unbalanced phenolic composition, has been evaluated by means of HPLC-DAD-MSn, spectrophotometric measurements (color contributions of free anthocyanins, co-pigments and polymerized pigments) and tristimulus colorimetry (CIELab parameters). Also, the effect of this addition on the perceived astringency has been evaluated by a sensory panel. Furthermore, the influence of the composition of the CWM (obtained from red and from white grape skins) in the observed effects has been studied. The results obtained showed that the addition of CWM importantly affect both flavanol and anthocyanin composition and evolution, thus affecting wine color and astringency.

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## III.P.24

**MICROWAVE ASSISTED EXTRACTION AND ULTRASOUND ASSISTED EXTRACTION APPLIED TO THE PREFERMENTATIVE MACERATION OF RED WINES****Remedios CASTRO**

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**Keywords:**

prefermentative maceration, microwaves, ultrasounds, red wine

Prefermentative maceration in red wines is a process that is carried out in order to extract a higher amount of polyphenols from grape solid parts and to improve the final wine's organoleptic characteristics. This stage implies a long period of time that could be shortened with the employment of certain accelerating energies.

Techniques such as Microwave Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE) could be a good option to produce an acceleration of the maceration stage, which would facilitate the winemaking process in the production of red wines.

In this work the use of two accelerating energies MAE and UAE have been tested in the fermentative maceration of red wines. For comparison purposes, a traditional maceration was also carried out. In all cases, after maceration, musts were submitted to alcoholic and malolactic fermentation. All the samples (initial, after alcoholic fermentation and final wines), were submitted to chemical and sensory analysis. Total anthocyanins, total tannins, total polyphenols and color parameters were considered. Volatile compounds were determined by SBSE-GC/MS, whereas polyphenols were analyzed by UPLC/DAD. Several sessions of sensory analysis were also carried out in a standard tasting room.

Both MAE and UAE favored the extraction of volatile and polyphenolic compounds from solid parts, with a significant higher content in tannins for MAE samples. From a general point of view, MAE produced wines which presented good sensory profiles, whereas UAE wines exhibited certain organoleptic defects.

III.P.25

## MICROWAVE ASSISTED EXTRACTION APPLIED FOR THE ACCELERATED AGEING OF RED AND SWEET WINES

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**Keywords:** Accelerated ageing, Microwaves, Oak chips, Wine

Wood ageing is a process in which a young wine can be transformed into a higher quality and more appreciated product thanks to the extraction of chemical compounds from wood, among other aspects. However, this is one of the most expensive processes in the production of high quality wines, mainly due to the long periods of time employed. Therefore, a reduction of ageing time could be interesting from an economic point of view. Techniques such as Microwave Assisted Extraction (MAE) could be a good alternative for it.

In this work the joint use of two types of oak chips (treated with a hydro alcoholic solution and without treatment) and MAE was employed for the accelerated ageing of red and "mistela" sweet wines. MAE procedure was optimized by means of an experimental design and it was also compared to UAE. In addition, the same products were traditionally aged in 16 L American oak casks. All the samples were submitted to chemical and sensory analysis. Volatile compounds were determined by SBSE-GC/MS, whereas polyphenols were analyzed by UPLC/DAD. The sessions of sensory analysis were carried out in a standard tasting room.

Optimal MAE conditions were 5 g/L of chips for 10 minutes of extraction at 60 °C and 350 watts with constant stirring. These conditions favored the extraction of volatile and polyphenolic compounds from oak chips. It affected the organoleptic profile of aged wines, producing similar characteristics to those obtained with traditional ageing.

The treatment of the chips with hydro alcoholic solution reduced significantly the extraction of polyphenolic and volatile compounds, but compared to UAE, MAE extracted a higher amount of volatile compounds and produced wines with better sensory characteristics.

## III.P.26

## PEF TREATMENT OF MUST AND WINE FOR ON-LINE CLARIFYING AND FINING

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**Keywords:** PEF, colloidal stability, fining, continuous system

PEF application in winemaking mainly concerns the extraction of phenolic compounds from grape skins for red wines production. PEF-mediated fermentation control has also been tested with interesting results, and a considerable part of information has been collected on enzymes. There is a lack of data concerning the efficiency of PEF on the stability of chitinases and thaumatin-like proteins and, thus, on the application of PEF for wine fining purposes. This work aimed to test whether the application of PEF could trigger the cascade of phenomena that lead to the formation of haze in wines and if the altered condition allows an in-line filtration system to remove the aggregates and to stabilize the product. Parallel investigations of the stability of the color studied any possible deleterious consequence on the quality. The samples used for the study were 2016 *Vitis vinifera* L. cv Moscato bianco must and *Vitis vinifera* L. cv Sauvignon blanc wine. PEF treatments with monopolar square wave pulses were performed in a lab-scale continuous system. The must and wine samples were pumped at 25 L/min into a PEF treatment chamber with 1 cm distance between the stainless-steel electrodes. Acquisition of voltage and current was via a digital oscilloscope. Two different experimental plans were devised, one for the Muscat must and one for the Sauvignon blanc wine. On Muscat must the PEF treatment consisted of two different trials with a total input energy of  $W=100$  kJ/ and  $W=150$  kJ/kg, respectively. With regard to Sauvignon blanc wine, an additional trial with a cooling step subsequent to the heating induced by the PEF treatment was applied. At the inlet and outlet of the PEF system thermocouples recorded temperature every second. Sixteen hours after the treatment, the samples were filtered on  $0.45 \mu\text{m}$  membranes and stored at  $20^\circ\text{C}$  for turbidity and color determinations up to 12 months. The results outlined that a combination of PEF treatments and membrane filtration reduced the Sauvignon blanc wine heat instability. Conversely, the Muscat must a significant increase of  $\Delta_{\text{NTU}}$  suggesting the inefficiency of the PEF treatments, possibly induced by the interaction with polysaccharides. As to the effects on color, Muscat must did not show any significant variation, while Sauvignon blanc wine showed a significant degree of pinking that disappeared 12 months after the PEF treatments.

### III.P.27

## THE USE OF CHITOSAN DURING WINEMAKING OF AGLIANICO GRAPES INCREASES THE PROPORTIONS OF FREE VERSUS BOUND SO<sub>2</sub> OF FINISHED WINES.

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**Keywords:** Sulfur dioxide, Chitosan, Wine fermentation, ratio free/total sulfur dioxide

The correct management of sulfur dioxide in wine industry is fundamental to preserve its quality. Initial additions of SO<sub>2</sub> to a newly fermented wine are typically on the order of 50 mg/L, and it is not uncommon that more than half of this addition to become bound SO<sub>2</sub>. The strongest SO<sub>2</sub> binder is acetaldehyde, while other carbonyl compounds, as pyruvic acid and ketoglutarate, are weak binders. Therefore, the amount of carbonyl compounds produced during alcoholic fermentation could influence the ratio free/total sulfur dioxide. For this reason, it could be interesting to evaluate the use of alternative antimicrobials that could replace or reduce the use of sulfur dioxide during the initial phases of vinification.

Among antiseptic used in admitted in winemaking chitosan has received considerable attention due to its antimicrobial activity against a broad range of microorganisms, such as bacteria, yeast and moulds. The antimicrobial action of chitosan against microorganisms was attributed to its positive charges that would interfere with the negatively charged residues of macromolecules on the cell surface, affecting membrane integrity.

In the present investigation fermentations of red musts either in the presence of chitosan or sulphur dioxide were carried out in order to compare the activity of both exogenous antimicrobial. The experimental plan provided for the vinification of the Aglianico grape by selecting two vinification batches, one made of 100% of healthy grapes and one composed of 50% with berries damaged by acid rot. For both batches six treatments were applied varying the timing of chitosan and SO<sub>2</sub> addition (before fermentation or just after fermentation). All resulting experimental wines were successively added with SO<sub>2</sub> to have a free level of 25±3mg/L.

At the end of the fermentation, no significant differences among experimental wines were found regarding yeast loads (total yeasts, non-*Saccharomyces* and cycloheximide resistant yeasts). Conversely, the ratio free /total sulfur dioxide of finished wines were different. In all the samples treated with chitosan a lower content (17-33% less) of total sulfur dioxide was detected while free SO<sub>2</sub> ranged between 24,96 to 27,94 mg/L. Since in all wines treated with chitosan a lower amount of free + α-hydroxysulfonates carbonyls (2-ketoglutaric acid, pyruvic acid, acetoin and acetaldehyde) were detected, these results indicate that the use of chitosan during winemaking can improve the proportions of free versus bound SO<sub>2</sub>.

## III.P.28

## TOASTED VINE-SHOOTS USED AS OENOLOGICAL ADDITIVES INCREASE THE VANILLIN AND TRANS-RESVERATROL CONTENT OF WINES

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**Keywords:** trans-resveratrol, vanillin, vine-shoots, wines

The interest for the revaluation of agricultural wastes had led to search new alternative uses for vine-shoots (v-s), the principal vineyard pruning waste, which could be a promising possible bioresource of bioactive compounds with innovative potential applications.

The chemical composition of vine-shoots has been widely studied in terms of volatile and non-volatile phenolic compounds. Especially significant is the increment of trans-resveratrol and vanillin while vine-shoot post-pruning storage for 6 months and when they are toasting. Added to the wines, it was observed that in Tempranillo red wines, the flavoring compound vanillin was found in a concentration four times above its odor threshold and in Airén wines vine-shoot, the trans-resveratrol concentration was close to the ones obtained for red wines. These results confirmed us the possibility of use vine-shoots as enological additives to modulate wine aroma due to vanillin and enhance the functional properties of wines for its increase in trans-resveratrol.

However, since these compounds are present in vine-shoots in higher concentration than those found in wines and that the contribution of each vine-shoots variety was different, the objective of the present work was elaborated Tempranillo wines with a higher dose of their own vine-shoots, and on the other hand, winemaking Tempranillo wines with Airén vine-shoots. Toasted vine-shoots of Tempranillo and Airén in two formats were added (12g/L): a) In alcoholic fermentation (Tempranillo v-s), removing them at the end of the wine-making process and adding them to macerate on month. b) After malolactic fermentation (Airén v-s) and macerating one month. The content of trans-resveratrol and vanillin was analyzed with HPLC-DAD and SBSE-GC-MS. Vanillin reached levels close to 250 µg/L in wines elaborated with chip format and near 300 µg/L when granule format was used. Sensory analysis supported these results, wines were described as silky with a distinctive positive profile. In the case of trans-resveratrol, levels of this compounds increased in wines until 2.05 mg/L when Airén chips were used and, surprisingly, in wines macerated with Airén granule trans-resveratrol concentration reached levels of 8 mg/L, which could enhance the functional properties of this wines.

As conclusion, the addition of vine-shoots to wine can be a new and innovative alternative for these waste, connecting viticulture and enology, in a new concept for "Circular Viticulture".

### III.P.29

## ULTRASOUND ASSISTED EXTRACTION APPLIED FOR THE ACCELERATED AGEING OF RED AND SWEET WINES

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**Keywords:** Accelerated ageing, Ultrasounds, Oak chips, Wine

Ageing in wood is a traditional practice usually employed to give to the wine certain physicochemical and sensory properties. In this way, a young wine is transformed into a higher quality and more appreciate product. However, this process implies a high cost and long periods of time. Techniques such as Ultrasound Assisted Extraction (UAE) could be a good option to produce an acceleration of the ageing process, which would entail a reduction of the costs in the production of wines.

In this work the joint use of two types of oak chips (treated with hydro alcoholic solution and without treatment) and ultrasonication was employed for the accelerated ageing of red and "mistela" sweet wines. Two different procedures (static and dynamic UAE) were tested and optimized by means of experimental designs. For comparative purposes, the same products were traditionally aged in 16 L American oak casks. All the samples were submitted to chemical and sensory analysis. Volatile compounds were determined by SBSE-GC/MS, whereas polyphenols were analyzed by UPLC/DAD. The sessions of sensory analysis were carried out in a standard tasting room.

On the one hand, the optimal conditions for static UAE were the use of ultrasonication for 1 hour employing pulses of 40 seconds ON/20 seconds OFF and an amplitude of 40%. On the other hand, the optimization for dynamic UAE led to 3 days of extraction with pulses of 1 minute ON/2 minutes OFF.

UAE produced significant variations of the aged wines, and favored the extraction of volatile and polyphenolic compounds from oak chips. It affected the organoleptic profile of aged wines, producing similar characteristics to those obtained with traditional ageing. Static UAE extracted a higher amount of polyphenolic and volatile compounds and produced aged wines with a better sensory profile, mainly when treated chips were employed. However, dynamic UAE produced negative sensory effects in some cases.

The treatment of the chips with hydro alcoholic solution reduced significantly the extraction of polyphenolic and volatile compounds and those without treatment produced sensory defects in some aged wines.

**III.P.30****UNDEREXPANDED SUPERSONIC CO<sub>2</sub> FREEZING JETS DURING CHAMPAGNE CORK POPPING****G rard LIGER-BELAIR**

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**Keywords:** champagne, cork popping, adiabatic expansion, carbone dioxide

During champagne cork popping, the CO<sub>2</sub>/H<sub>2</sub>O gas mixture initially under pressure in the bottleneck freely expands into ambient air and experiences adiabatic cooling. A comparison between the condensation phenomena accompanying cork popping from bottles stored at 20 and 30  C was made. The initial headspace-to-ambient-pressure ratio much exceeded the critical ratio needed for the gas mixture to reach Mach 1, thus forming under-expanded supersonic jets expelled from the throat of the bottlenecks.

Unlike the deep blue haze observed for the bottles stored at 20  C, the plume freely expanding from the bottles stored at 30  C turned grey-white. It was emphasized that, after adiabatic cooling and with a saturation ratio for gas-phase CO<sub>2</sub> about twice higher for the bottles stored at 30  C, dry ice CO<sub>2</sub> clusters grow bigger and reach the critical size needed to achieve the Mie scattering of light. Moreover, during the very first millisecond following the cork popping process, evanescent normal shock waves (or Mach disks) were unveiled in the plumes, until the reservoir-to-ambient-pressure ratio goes below a critical ratio needed for the formation of Mach disks, which were de facto seen vanishing.

### III.P.31

## USE OF LINEAR VOLTAMMETRY TO FOLLOW ROSÉ PRESSING

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**Keywords:** Electrochemistry, Voltammetry, Oxydation, Color of rosé

In oenology, electrochemistry offers many fields of research. The voltammetry consists of sweeping the electrical potential of the sample and to measure the intensity of the current generated by the release of electrons by the antioxidant compounds when they are at their potentials. The total amount of electrons (and thus the sum of the generated electric current) and the shape of the curve linking the potential and the current provide very interesting information to globally characterize the ability of the sample to resist oxidation.

We have developed and used this technique notably to have a tool allowing to follow the operation of press during the winemaking. During this study we have done the analysis on Cabernet Franc and Tanat during their pressing. Two types of press were compared: one with open cage and another one with inert cage to investigate the impact of oxygen. At the same time, a chromatic analysis (L.a.b system) were done in order to evaluate the possible deviations in the hue of rosé wines according the electrochemistry profiles.

Free-run juices have shown good electrochemical stability whereas press wines have presented an important heterogeneity. A difference between the beginning and the end of the pressing has been noticed. The frequency and number of the press rotation have also played an important role. These observations have permitted to our partnership to valid the best way of pressing and provide also interesting data to choose the best treatment after the pressing (SO<sub>2</sub>, fining...)

**III.P.32****POLYVINYLPOLYPYRROLIDONE FINING DURING FERMENTATION: EFFECT ON POLYPHENOLS AND THIOL AROMA CONTENT****Mélodie GIL (SPEAKER : C. SAUCIER)**

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**Keywords:** Rosé wine, PVPP, Phenolic compounds, Thiols

During winemaking, one way to adjust rosé wine color and prevent possible organoleptic degradations involves Polyvinylpolypyrrolidone (PVPP) fining.

The impact of PVPP treatments on rosé wine color was measured by colorimetry, and the resulting decrease in color was directly related to pigment adsorption.

The polyphenol composition before and after fining was followed and quantified using UPLC-ESI-MS/MS. Three polyphenol families had a specific adsorption affinity towards PVPP. Anthocyanins and flavonols which are the red and yellow wine pigments were mostly adsorbed, and a specific adsorption of coumaroylated anthocyanins compared to the other anthocyanins was observed. Uncolored flavanols were also preferentially adsorbed which may reduce future oxidation and browning problems.

The thiol content was also investigated. Compared to the control, an increase of 3-mercaptohexyl acetate (3MHA) and 3-mercaptohexan-1-ol (3MH) was observed depending on the PVPP dose applied. Our hypothesis is that fining removes polyphenols, thus reducing the formation of oxidation products like quinones known to trap thiol aromas.



# Analysis and composition of grapes, wines, wine spirits

#### IV.P.1

### IMPACT OF OAK WOOD BARREL TANNIN POTENTIAL AND TOASTING ON WHITE WINES ANTIOXIDANT STABILITY

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**Keywords:** ellagitannins, glutathione, sulfur compounds, radical chemistry

Wines aged in oak wood barrels with various uniform tannin contents (which were classified according to their total ellagitannins contents as predicted by Near Infrared Spectroscopy on the untoasted wood) and different toasting levels (high precision toasting by radiation) were distinguished according to their overall abilities to resist against oxidation. Wine trials were carried out on two different vintages (2015, 2016) and three grape varieties (Sauvignon blanc, Sémillon, Chardonnay). Regardless of the vintage and the wine matrix, a positive correlation was established between wines oxidative stability (based on EPR spin trapping methodology) and oak barrel tannin potential. The extraction kinetic of ellagitannins by wines appeared linear during barrel aging and achieved its maximum at six or eight months, in a grape variety dependent manner. Oak wood barrel tannin potentials and toasting had no effect on wines glutathione and polyphenols contents. However, wines aged in new barrels with both low and medium tannin potentials, preserved at the end of aging and important number of S-N containing compounds, which in addition to the known ellagitanins revealed wines better antioxidant stability.

## IV.P.2

## INFLUENCE OF PREHARVEST ELICITORS TREATMENT TO MONASTRELL GRAPES ON COMPLEX CARBOHYDRATES OF WINES

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**Keywords:** grape, wine, elicitors, complex carbohydrates

The elicitors application impacts on the phenolic composition of grapes and their corresponding wines [1]. But an increase in phenolic content of treated-grapes is not always translated into wines [2]. The use of elicitors can also cause changes on the cell wall of berry skin, increasing its rigidity. The phenolic extraction from grape to wine but also the release of polysaccharides and oligosaccharides could be affected. These two macromolecules affect sensory properties in wines [3].

The aim of this work was to evaluate the effect of preharvest application in Monastrell berries of 4 different elicitors (benzothiadiazole -BTH-, methyl jasmonate -MeJ-, chitosan from fungi -CHSf- and chitosan from seafood -CHSs-) on wine polysaccharide and oligosaccharide fractions. These two fractions were isolated according to the method previously described [4]. Polysaccharide composition was estimated from the concentration of individual glycosyl residues determined by GC-MS [5].

Sugar composition of oligosaccharides was determined after solvolysis by GC of their per-O-trimethylsilylated methyl glycoside derivatives [6].

The total polysaccharide concentration reduced when BTH, MeJ, CHSf and in particular CHSs were applied on the grapes. Polysaccharides rich in arabinose and galactose showed lower content after the 4 studied treatments. Mannoproteins amount was also higher in control wine compared to wines elaborated with the 4 types of treated-grapes, whereas wine from CHSs treated-grapes presented the lowest concentration of rhamnogalacturonan II. Moreover, all the studied treatments affected the proportion of different polysaccharide families. On the other hand, both CHSs and CHSf elicitors decreased the total oligosaccharide amount of their corresponding wines. Finally, wines elaborated with CHSs treated-grapes exhibited the highest value of the characteristic [(Arabinose + Galactose) to Rhamnose] ratio from oligosaccharide composition, suggesting more structures from the hairy regions of pectins.

It can be deduced that the separate use of MeJ, BTH, CHSf and particularly CHSs induces a lower degradation of cell wall from grape skin compared to control sample. It could be due by a reinforcement of the skin cell wall through the action of these 4 elicitors. Therefore, the application of any of these 4 elicitors in the vineyard's clusters influenced the complex carbohydrate composition of elaborated wine, which could impact on its sensory properties.

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#### IV.P.3

### DETECTION OF MARKERS FROM PREMATURE WINE OXIDATION (OR RANDOM OXIDATION) OF WHITE WINES CLOSED WITH CORK STOPPERS

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**Keywords:** wine, oxidation, cork, closure

To date, cork is the most important material for wine bottles closures: it is sustainable and offers to users the most natural way to seal wine. Despite its specific physicochemical properties, the variability among natural cork stoppers is a controversial issue because of random or premature wine oxidation (POx) cases. This phenomenon occurs when a wine with aging potential is found to be oxidized and often undrinkable. POx markers identification, reliable methods for their detection in wine, and correlations between POx markers and the system "wine-bottle-closure" (WBC) are still unknown. This paper aims at detecting POx markers in 5 white wines bottled with natural corks, and stored upright under controlled temperature and humidity conditions, and to search for relationships between POx and WBC. To detect POx markers, a non-targeted screening was performed on a metabolomics platform constituted of a hybrid quadrupole-time-of-flight mass spectrometer coupled to a UHPLC chromatographic system. Investigations on WBC system included the analyses of sulfur dioxide, volatile acidity, acetaldehyde, color, appearance and aroma, physical and mechanical parameters of stoppers, headspace and bottle neck inner profile. Statistically, the analytical data were treated with ANOVA, fold-change analysis, PCA and PLS-DA. Firstly, ANOVA results from metabolomics analysis outlined the grouping of the samples depending on wine type, and within each one, separation according to the POx intensity (low-medium-high oxidation level) was evidenced. Then, PLS-DA performed using all the data highlighted three homogeneous groups each comprising the different wines and listing those compounds with a significant discriminating capacity toward POx intensity. Finally, correlations were attempted between the POx intensity and the parameters belonging to the WBC system.

**IV.P.4****PHENOLIC PARAMETERS EXPLAINING DIFFERENT ASTRINGENCY PROPERTIES IN RED WINES****Angelita GAMBUTI**

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**Keywords:** tannins, Italian wines, astringency, correlations

The astringency perception of wine tannins is not related only to the astringency global intensity but several sub qualities have to be considered. Descriptors as complex and velvet are associated to a pleasant sensation while attributes as drying, harsh and unripe are representative of negative astringency features. Several instrumental methods involving tannin precipitation were developed in order to objectively estimate wine astringency but up to now none of them is able to predict all aspects of astringency perception. In this study we investigated the link between methods usually used to characterize wine tannins and phenolics and astringency features. Seventy-five wines belonging to 11 Italian grape varieties were evaluated by 14 wine experts for different levels of global astringency GAs, sum of positive attributes (complex and surface smoothness) PAs and, sum of negative attributes (drying, harsh and dynamic) NAs. A set of chemical parameters significantly correlated ( $\alpha=0.05$ ) to astringency sub-qualities was individuated: GAs are positively correlated with total proanthocyanidins, VRF (vanillin reactive flavans), MCP (methyl cellulose precipitable tannins), BSAreactive-tannins and SPI (saliva precipitation index); NAs are positively correlated with total proanthocyanidins, VRF, BSAr-tannins, SPI, MCP and negatively with large polymeric pigments (LPP) and anthocyanins/BSAreactive-tannins ratio (A/T); PAs are positively correlated with LPP, A/T and negatively correlated with total proanthocyanidins, VRF and BSAreactive-tannins. Tannin concentration estimated by phloroglucinolysis was positively correlated with GAs and NAs while a negative correlation with PAs was observed. Total wine polysaccharides and proteins are not significantly correlated with any of the measured astringency parameters. Each astringency predictive methods based on tannin precipitation assays is explained by different chemical variables: BSAreactive-tannins by total proanthocyanidins, total phenolics, VRF and  $Abs_{280nm}$  ( $R^2=0.9164$ ); MCP by  $Abs_{280nm}$ , total phenols, total proanthocyanidins, vanillin reactive flavans and  $Abs_{230nm}$  ( $R^2=0.8151$ ); SPI by LPP,  $Abs_{230nm}$ , total proanthocyanidins and vanillin reactive flavans ( $R^2=0.4501$ ).

Acknowledgements: MIUR project N. 20157RN44Y. P. Arapitsas<sup>3</sup>, V. Gerbi<sup>4</sup>, M. Marangon<sup>2</sup>, A. Versari<sup>5</sup>, A. Ricci<sup>5</sup>, S. Rio Se-gade<sup>4</sup>, L. Rolle<sup>4</sup>, B. Simonato<sup>6</sup>, G. Tornielli<sup>6</sup>, S. Vincenzi<sup>2</sup>

#### IV.P.5

### SELECTIVE SORPTION OF HALOANISOLES AND HALOPHENOLS FROM CONTAMINATED WINE INTO ALIMENTARY FILM

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**Keywords:** TCA, alimentary film, phenolic composition, sensory analysis

The mouldy off-flavour in wine commonly called cork taint is a serious quality-related problem for wine industry. Up to now no treatment exists to eliminate the presence of these unpleasant volatiles in wine. Thus, this research aimed i) to assess the efficacy of the alimentary film to remove or lessen HAs content in polluted wines; and ii) to evaluate its impact on wine quality (phenolic/aromatic profile, sensory attributes). For this reason, red wine aged in barrels (A, B, C) with a different level of haloanisoles (HAs) pollution was investigated. After 24 months of ageing, wine was treated with an alimentary film and sampled after 8, 24 and 48h of wine-film contact.

The film-treatment reduced significantly ( $p < 0.05$ ) the TCA content of initial wine. This decrease became more noticeable as the contact time film-wine increased. After 8h of wine-film contact, TCA concentration lessened up to 47-57% of the initial content. A longer treatment of 24h and 48h led to a reduction of 73-75% and 81-83% of TCA contamination, respectively.

Chromatic characteristics, phenolic and proanthocyanidin contents of wines did not change because of the film treatment. Furthermore, its use did not impact significantly the anthocyanidin content of wines up to 24h of film-wine contact.

Woody aroma profile of wines from barrels A and C remained constant during film treatment. For barrel B, vanillin (13-28%) content decreased in wines beyond 8h of film treatment, but remained at concentrations above its perception threshold (320  $\mu\text{g/L}$ ) after the film treatment. Regarding the fruity aroma profile, only a significant reduction of the main ethyl esters of straight-chain fatty acids was observed (6-65%, 18-64% and 21-82% after 8h, 24h and 48h of film-treatment, respectively) ( $p < 0.05$ ).

Sensory panel significantly distinguished between contaminated initial wines and film-treated wines after 8h, 48h and 24h of film contact for barrels A, C and B, respectively. They were all significantly perceived as less corky, more fruity and woodier than the corresponding initial untreated wines. Judges' perception was linked to the TCA contamination reduction after film-treatment, which was acting as masking agent of pleasant notes.

Thus, the alimentary film was able to improve the organoleptic quality of wines contaminated with HAs, by reducing the corky notes and increasing their overall woody and/or fruity aromas, without highly impacting their chromatic parameters, phenolic and aromatic composition.

## IV.P.6

## EFFECT OF PRE-FERMENTATIVE STEPS ON THIOL PRECURSORS IN GRILLO MUST

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**Keywords:** Thiol precursors, Grillo grape, Pressing, UPLC-HRMS

The varietal thiols, namely 3-mercaptohexan-1-ol (3MH), 3-mercaptohexylacetate (3MHA), and 4-mercapto-4-methylpentan-2-one (4MMP), are sulphur-containing aromas associated with the typical flavour of several white wines, such as Sauvignon blanc wine, conferring guava, citrus and passion fruit notes. These compounds occur as non-volatile sulphide precursors in grape berry, where they share their sulphur atom with a cysteine residue. 3MH bound with cysteine (Cys-3MH), glutathione (GSH-3MH) and also cysteine-glycine (CysGly-3MH) has been described, while 4MMP occurs as cysteine (Cys-4MMP) and glutathione (GSH-4MMP) conjugates. S-3-(hexan-1-yl)-glutathione (GSH-3MHA) was also identified, and it can be considered as a thiol precursor. Recently, the presence of thiol precursors was reported in the Italian autochthonous variety Grillo, and their concentrations strongly decreased when must was produced under commercial conditions.

This study investigated the influence of pre-fermentative operations on thiol precursor concentrations. Grillo grape was pressed under industrial conditions; must samples were collected after crashing, at draining, at pressing yield of 20%, 40% and 60%, at the end of pressing, during transfer in clarification tank, in a clarification tank and after clarification. The must was either air-exposed or air-free during the pre-fermentative steps. Thiol precursors were determined in SPE-purified must samples by UPLC-HRMS.

Cys-3MH, GSH-3MH and GSH-3MHA strongly decreased after crashing, and small concentrations were found in drained must samples independently of the presence of air. In particular, the crashing played a major role on GSH-3MHA content; a further decrease of both Cys-3MH and GSH-3MH was found due to the must transfer in clarification tank. In general, precursor amounts were lower in must samples produced in air-free condition, except for clarified musts where the precursor contents were comparable in both air-exposed and air-free conditions.

For the must production at industrial conditions particular attention should be given to the grape pressing for limiting the loss of thiol precursors. The air-exposure of must has a limited positive influence on thiol precursors, since the removal of solid parts with the clarification is responsible for their further loss. The proper management of these winemaking steps could allow to preserve major levels of thiol precursors.

#### IV.P.7

### INFLUENCE OF YIELD AND HARVEST DATE ON SHIRAZ WINE VOLATILE AND NON-VOLATILE COMPOSITION

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**Keywords:** GCxGC-TOFMS, LC-MSMS, metabolomics, secondary metabolites

Targeted and untargeted metabolomics was applied to wine samples in order to determine the effect of yield and harvest date on Shiraz wine volatile and non-volatile composition within the same warm-hot Australian mesoclimate. Vines were drip irrigated and trellised to open sprawling canopy with an average yield from 10.2-18.5 kg/vine/vineyard. Shiraz wines were made in triplicates from grapes harvested at two occasions 10 to 12 days apart (harvest 1; H1 and harvest 2; H2, respectively) commencing 12 days from the plateau of berry sugar accumulation.

Wine volatiles were acquired by HS-SPME-GCxGC-TOFMS. A total of 1,276 putative compounds were detected in at least one of the wine samples and 175 compounds showed significant trends related to grape maturity. The first two dimensions of the PCA accounted for 57% of the variation and separated the samples according to the harvest date, irrespective of the yield. Trained tasting panels were able to perceive differences between wines from H1 and H2.

Wine polyphenols and wine pigments were quantified by LC-MS/MS. Vineyard yield had a predominant effect on wine color related pigments, whereas harvest date was of lesser importance. More than 50 quantified polyphenols in wines were poorly correlated with either harvest date or yield.

In conclusion, common evolution of wine volatiles, irrespective of site particularities was noticed for Shiraz, whereas it seems that wine non-volatile composition (colour related compounds and polyphenols) is at bigger influence of site rather than harvest date in the late ripening.

## IV.P.8

## TWO DIFFERENT DATES OF APPLICATION OF ELICITORS TO MONASTRELL GRAPES: IMPACT ON THE COMPLEX CARBOHYDRATES OF THEIR WINES

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**Keywords:** methyl jasmonate, benzothiadiazole, polysaccharides, oligosaccharides

Monastrell cultivar is the main winemaking grape in the Denomination of Origin Jumilla (SE Spain). Different strategies are being carried out to improve the quality of the Monastrell wines in order to improve in quantity and quality its polyphenolic content, and at the same time respecting the ecosystem. In this sense, the application of elicitors such as methyl jasmonate (MeJ) and benzothiadiazole (BTH) has given satisfactory results in the increase of polyphenols of different grape cultivars, as well as inducing resistance to certain diseases. However, a difficulty has been described in the extraction of its phenolic compounds from grapes to wine, due to variations in different components of the skin cell wall. Some of these components, such as polysaccharides and oligosaccharides released into wine, depend on the nature of the cell wall, and may be affected. The role of these complex carbohydrates in organoleptic and physico-chemical properties of wine has been largely demonstrated.

The aim of this study was to check if separated application of MeJ and BTH, as well as their combined addition (MeJ+BTH) during two points of the maturation cycle (veraison and mid-ripening) of Monastrell grapes, influenced the amount and composition of complex carbohydrates (polysaccharides and oligosaccharides) released in their wines. The results obtained in two consecutive years (2016 and 2017) revealed a lower concentration of polysaccharides and oligosaccharides in the elaborated wines from treated grapes, particularly when treatments were applied during veraison. Therefore, it can be suggested that the application of these elicitors affected the characteristics of the cell walls from berry skins, which consequently would impact on the elaborated Monastrell wines. From these results, it can be concluded that the response from the berries to the elicitor treatments was a strengthening of their cell walls, and consequently the activation of defence mechanisms can be hypothesized.

#### IV.P.9

## CHITOSAN APPLIED AS A GRAPEVINE BIOSTIMULANT MODULATES GRAPE COMPOSITION AND WINE QUALITY

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**Keywords:** biostimulant, chitosan, grapevine, wine

The extensive use of chemicals for grapevine protection is associated with environmental issues. Therefore there is an increasing trend towards complementary and/or alternative protection methods.

Biostimulants are compounds able to elicit the synthesis of secondary metabolites in plants leading to an increase of their natural defense mechanism. Some of these metabolites can impact wine sensory properties as color, mouthfeel and aroma. This project aims at understanding whether treating grapevines with a biostimulant (chitosan hydrochloride) could result in modifications detectable at plant, grape and wine levels. Sauvignon blanc vines were grown in pots in a glasshouse to avoid potential pathogen attacks so that the effects of the treatments would be solely attributable to the biostimulant. A total of 74 plants were used in a randomized block design, with each block containing a control and a treated set of vines. The vegetative growth of the vines, the chemical composition of the grapes and of the wines produced were assessed.

Results showed that the foliar application of chitosan did not affect the plant growth as no significant variation in shoot length was seen between treated and control vines. Additionally, no effects in polyphenol content of leaves was noticed. The treatment resulted in a significant decrease of the PPO activity (-65%), and a consequent increase in the antioxidant activity (+29 %) of grape berries due to their higher content of phenolic compounds (+14%). The protein content of treated grapes was significantly higher than control (+59%), and SDS-PAGE profiling showed that treated grapes contained significantly less thaumatin-like proteins and more  $\beta$ -glucanases than control grapes. At harvest, grapes from the two treatments were pooled, processed and the obtained juice was fermented in standard white winemaking conditions.

Wines made from treated grapes had significantly higher amounts of total polyphenols (+18%) and total polysaccharides (+35%), higher Abs<sub>320 nm</sub> values (+10%), and a slight increase in total proteins (+5%).

In general, chitosan application was able to significantly impact some of the secondary metabolites of the grapevines as phenolics, polysaccharides and proteins, all compounds exerting important technological and sensorial roles in wine. Further studies are needed to assess the effects of different biostimulants, on different varieties and in open field conditions.

Acknowledgements: project N.2017 – prot. BIRD179194; A Dupas de Matos, M Vegro, S Zannoni

## IV.P.10

## INFLUENCE OF GRAPES GEOGRAPHICAL ORIGIN AND YEAST STRAIN ON AROMA PROFILE OF CORVINA AND CORVINONE WINES

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**Keywords:** Aroma, Geographical origin, Yeast, Terroir

Geographical origin is a relevant factor for wine aroma, as it influences vine, modifying grape chemical composition and enabling discrimination of wines from different places. Complex interactions between different factors such as environment, grape variety and viticultural techniques means that each production site can potentially produce grapes with a unique chemical fingerprint (Van Leeuwen, *et al.*, 2004). However, less scientific literature is available on the relationship between such potentially unique grape chemical fingerprints and composition of the final wine in terms of metabolites potentially contributing to its perceived sensory characteristics and quality. We have previously reported significant differences in the volatile composition of single vineyard wines, highlighting an important contribution of either grape geographical origin, fermentation practices or ageing (Slaghenaufi & Ugliano, 2018; Luzzini, 2018).

This study aims to interpret the contribution of two main variables such as yeast strain and geographical origin of the grapes to red wine volatile composition and sensory characteristics. Experimental red wines were produced with Corvina and Corvinone grapes obtained from two different geographical areas within the Valpolicella region, fermented with four different commercial yeasts plus a spontaneous fermentation. In consideration of the local traditional practice of post-harvest withering of the grapes, wines have been obtained from either fresh and withered grapes. Experimental wines were analysed by SPE-GC-MS and SPME-GC-MS and sensory analysis (Sorting task). Multivariate analysis of the results of analytical techniques shows good differentiation for all the variables at the same time (cultivar, geographic origins and yeast strain). Differentiation attributable to yeast is observed, in particular in relationship to different contents of esters such as isoamyl acetate, ethyl octanoate, ethyl hexanoate, ethyl butanoate and ethyl 3-methylbutanoate, but also  $\delta$ -decanolactone and furfural. Differences due to geographical origin of grapes were mostly associated with vanillates and terpenes such as linalool and  $\alpha$ -terpineol. For Corvinone wines  $\beta$ -damascenone also differentiated geographical origin, while for Corvina wines  $\beta$ -citronellol also played a role. Sensory analysis by means of sorting showed that, in the case Corvinone wines, geographical origin had greater influence than yeast strain, whereas for Corvina wines none of the two variables prevailed over the other.

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#### IV.P.11

### STUDY OF VOLATILE ORGANIC COMPOUNDS OF SOUTH TYROLEAN PINOT BLANC MUSTS AND WINES USING SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-TIME OF FLIGHT-MASS SPECTROMETRY (SPME/GC-TOF-MS) ANALYSIS

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**Keywords:** Pinot blanc, Volatile Organic Compounds, aroma, SPME/CG-ToF-MS

The *Vitis vinifera* cultivar Pinot blanc is one of the most important grape varieties cultivated in South Tyrol (Italy). The Pinot blanc wines are characterized by a fresh-fruity aroma also given by their volatile organic compounds (VOCs). The organoleptic properties of white and red wines have been widely studied, leading to the identification of several volatile compounds which are responsible for characteristic wine flavours. However, scarce information is available concerning the volatile composition of Pinot blanc wines. Moreover, the increasing temperature in the Alps forces the winegrowers to harvest their grapes earlier in the season, leading to a negative influence on the peculiar aroma of Pinot blanc wines. For this reason, moving into higher and thus cooler vineyard locations represent a possible solution to ensure the quality of South Tyrolean Pinot blanc wines.

The aim of this project is to identify and chemically characterise the volatile composition of Pinot blanc musts and wines from several terroirs and altitudes.

Eight vineyards between 200 and 700 m a. s. l. in four South Tyrolean wine growing areas were investigated. Grapes were harvested in two different time points during season 2017 and subjected to the same vinification protocol. Harvesting and vinification were done in triplicate, resulting in 42 musts and wines. A solid-phase microextraction gas chromatography-time of flight-mass spectrometry (SPME/GC-ToF-MS) analysis was implemented to determine the VOCs which are responsible of the typical aroma of the South Tyrolean Pinot blanc. A total of 30 and 60 VOCs was detected in Pinot blanc musts and wines, respectively. Particularly, the most abundant VOC found in Pinot blanc wines is 1-butanol, 3-methyl which is the major higher chain alcohol in alcoholic beverages. Other major VOCs found in Pinot blanc wines are two ethyl esters, one primary alcohol and three fatty acids. On the other hand, four C-6 aldehydes and alcohols and one monoterpene commonly found in alcoholic beverages are the major VOCs found in Pinot blanc musts.

Finally, differences in terms of VOC abundance were detected among all wines from the different sites investigated. VOC amounts in wine samples from grapes harvested at high and low altitudes and at two different harvesting points deserve particular attention, suggesting that Pinot blanc aroma can be influenced by both terroir and altitude of cultivation.

*Acknowledgements:* The "PinotBlanc" project has received funding from the European Regional Development Fund, ERDF 2014-2020.

## IV.P.12

## CHEMICAL DESCRIPTION AND ORGANOLEPTIC EVALUATION OF PINOT NOIR WINES FROM DIFFERENT PARTS OF ITALY: A THREE YEARS' INVESTIGATION

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**Keywords:** Pinot Noir, LC-MS, sensory evaluation, tannin composition

Pinot Noir is an important international grape (*Vitis vinifera*) cultivar, with an area under the vine of ca. 112,000 ha worldwide. Therefore, it is one of the most widespread and important red wine grape cultivars [1]. In Europe, Italy is the fourth biggest producer of Pinot Noir (after France, Germany and Switzerland), with 5,046 ha of which 464 ha are located in South Tyrol[2, 3].

Pinot Noir is highly appreciated as a monovarietal grape for the production of both sparkling white (including Champagne) and still red wines: Pinot Noir is considered an “elegant” red wine because of its well-balanced structure with fine organoleptic features, making it suitable for mid- to long-term ageing.

In this work, a chemical description of several Pinot Noir wines from different parts of Italy is presented. Wines samples were taken from an Italian annual national Pinot Noir competition performed in three subsequent years (2016 - 2018). All wines were evaluated by a tasting panel, using the overall sensory quality as the only descriptor. Most wines were registered for the competition the same year they were put on the market. Moreover, all of them were 3-years old from the vinification at the moment they were analyzed and evaluated.

For the chemical screening standard oenological chemical parameters (total acidity, colour, alcohol degree, total phenolic content, tannin index, etc.) and the content of the most abundant phenolic molecules were used. The latter were obtained by means of LC-MS analysis. For quantification of target phenolic compounds in wine, LC-MS triple quadrupole with internal standards was used. The compound identity was confirmed by retention time (Rt) and multiple reaction monitoring (MRM) using a minimum of two transitions per molecule.

A correlation between the chemical parameters with the tasting panel results was attempted; in particular, a correlation between tannin content and sensorial value was investigated. As a result, we found that polyphenols and tannin composition were highly variable among the different Pinot Noir wines and had a strong impact on their overall sensory evaluation.

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IV.P.13

**DIFFERENTIATION OF DISTILLED SPIRIT (LAMBANOG) FROM PHILIPPINE NIPA PALM ACCORDING TO GEOGRAPHICAL ORIGIN BASED ON METAL PROFILE OF RAW PALM SAP**

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**Keywords:** metals, lambanog, linear discriminant analysis, FAAS

There is a dearth of scientific investigations on Philippine traditional wines and spirits. Moreover, with the opportunities in local and global markets, it is imperative that the traditional wine and spirits industry of the country put premium on quality and consumer safety for it to benefit from such opportunities.

This study on the determination on heavy metal content of lambanog, a distilled spirit, sourced from the sap of *Nypa fruticans*, endeavors to fill gaps in empirical studies on Philippine alcoholic beverages.

Lambanog-making has long been one of the main livelihoods in Quezon, a province southeast of Manila, owing to the prolific growth of nipa palm in a number of its municipalities. The concentrations of 9 heavy metals (Fe, Zn, Cd, Ni, Pb, Mn, Cu, Co and Cr) were determined using flame atomic absorption spectrophotometry after dry mineralization and wet digestion of the distilled spirit, lambanog, and the raw, and fermented palm sap. Metal content profile in the three stages of lambanog production was established with the initial objective of identifying gaps in the manufacturing process. The results obtained from the metal profiling studies made possible the differentiation of the distilled spirits by geographical origin based on the respective heavy metal profiles of their raw nipa sap. Three municipalities that have extensive nipa palm plantations, situated along the northeastern Philippines Pacific seaboard, Mauban, Infanta, and Polillo Group of Islands, were selected as sampling sites. A total of forty-one (41) samples were collected from these municipalities. Leave-one-out cross validation was used to check the accuracy of a model prediction in linear discriminant analysis (LDA). The model correctly predicted the location of 63.41% of the distilled spirits. For each specific location, the percentages of correctly classified beverages are 70% from Infanta, 58.33% from Mauban, and 55.56% from Polillo. The mean concentrations of the metals ranged 0.302 to 3.3145 ppm for Fe, 0.081 to 6.33 ppm for Zn; meanwhile for Cd, 38 samples were below the detection (BDL) of 0.015 ppm except for three samples that contained 0.019 to 0.047 ppm; for Ni, 34 samples were below the detection limit of 0.029 ppm and only 7 samples had concentrations of 0.041 to 0.280 ppm; for Pb, 20 samples were BDL of 0.038 ppm while values ranged from 0.015 to 0.160 ppm for 21 samples; for Mn 4 samples were BDL of 0.004 ppm while 37 samples contained 0.008 to 1.439 ppm; for Cu only 1 sample was BDL of 0.051 ppm while the rest of the samples contained 0.086 to 0.480 ppm; all samples were BDL of 0.047 ppm and 0.020 ppm for Co and Cr, respectively. With such results, it is not only possible to identify the geographical origin of a lambanog sample as a function of its heavy metal profile but as well as identify intervention measures to improve lambanog manufacturing in the Philippines to meet international standards for maximum allowable limits for heavy metals.

## IV.P.14

## RIESLING WINES FROM UKRAINE: SPECIFICS OF THE REGIONAL WINES

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**Keywords:** Riesling, Ukraine, climate, analysis

Riesling (Rhine Riesling) is an aromatic grape variety with a noticeable acidity. It is suitable for the production of different styles of still and sparkling wines of high quality. Being strongly "terroir-expressive" variety Riesling reveals its variable character in wines depending on the region. The biggest plantations of Riesling are located in Germany, about 22500 ha (Anderson, 2013). The other important Riesling producing countries are USA (- 4850 ha), Australia (- 4100 ha), France (- 3500 ha) and Austria (- 1850 ha). At the same time, little is known about Ukraine with around 2700 ha of Riesling vineyards, which positions Ukraine on the 5th place in the world by the Riesling vineyards area.

The current project aimed to discover particularities of Ukrainian Riesling wines; to reveal how the local climatic conditions and winemaking practices influence composition of the wines. Most of the Riesling vines in Ukraine are cultivated at warmer conditions compared to German regions. Therefore, this project can be useful also for the future strategies of adaptation of German Riesling wines to the global warming and climate changes.

16 Riesling wines from Ukraine were selected and analyzed both in Ukraine and in Germany (sensory analysis, chemical analysis: general wine analysis, volatile compounds etc). 14 wines were originated from the south of Ukraine, where the main vineyards areas are located: Odessa, Mykolaiv and Kherson regions. Being on the 46th–47th parallels, mild and warm climate in these regions is influenced by the proximity to the Black Sea. Two other wines were from the smaller Zakarpattia region near the Carpathian mountains, between 48th and 49th parallel, similar to Baden-Württemberg German region.

Most of the studied Ukrainian wines were dry with residual sugar < 4 g/L; total acidity between 5 and 6.5 g/L; alcohol content ≤ 13% v/v. Because of the free SO<sub>2</sub> content regulations, these values were relatively low compared to German wines, often ≤ 10 mg/L. Effect of free SO<sub>2</sub> limitations would need further investigations also in the light of the global trends of SO<sub>2</sub> lowering in wine. Sensory evaluations showed that German wines were often perceived fresher with more pronounced citrus notes, while Ukrainian wines possessed more developed aromas. Some Ukrainian wines of 2016–2017 vintages had higher terpenes content than others, while aged samples from 2012–2014 possessed elevated level of vitispirane and 1,1,6-trimethyl-1,2-dihydro-naphthalene (TDN).

#### IV.P.15

## RUM AND COGNAC AROMA CHARACTERIZATION BY GC-FID AND PCA FOR PRODUCTS COMPARISON PURPOSE AND DEEPER UNDERSTANDING OF THE COMPOSITIONS OF BOTH SPIRITS

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**Keywords:** Cognac, Rum, Aroma, Composition

While sharing similarities, strong sensory and chemical differences exist between cognac and rum. They are likely to be due to the specificities throughout the spirit making processes, but are not fully understood. This work was performed to compare cognac and rum on the basis of their aroma composition and explain their distinctive characteristics.

Forty-four cognacs and 46 rums were sampled. The spirits were analyzed by direct injection in GC-FID. The molecules listed in EU regulation 2870/2000 (volatile substances content) plus a few others were quantified. PCAs were performed on the data.

The bi-plots show the clear separation between cognacs and rums along the first axis. Some of the main compounds correlated with the cognac samples are long ethyl esters, ethyl lactate, heavy higher alcohols, methanol, isobutanol or TDN. Isobutanol and methanol are more likely to be located nearby the youngest cognacs whereas esters and alcohols are more present in the oldest samples. Indeed, the cognacs are scattered along the second axis according to their age.

Some variables collinear with the rums are formiate, acetate and butanoate ethyl esters, isoamyl acetate, 1-propanol or 1- & 2-butanol. No clear scattering of the rums according to their age is visible but a group of products of the same origin, correlated with ethyl butanoate, is plotted away from the other samples along the third axis.

**The above methodology is suitable for the comparison of cognac and rum aroma compositions, beside a certain characterization of age and origin. It might be used as a predictive tool for unknown samples.**

The correlation of methanol and TDN to cognacs can be explained by the higher concentration of their respective precursors in grapes.

The higher ethyl lactate level in cognac suggests that the malolactic fermentation is more active than in rum.

The lighter esters are more concentrated in rum whereas the heavier are in cognac, probably favored by the distillation.

A specific rum, aged in a French oak cask (generally used for cognac), tends to get closer to the cognac samples. Meanwhile the same rum, aged in American oak (classic rum cask), is clearly located among its peers. This suggests that the typicity of both spirits is also strongly influenced by the type of cask during aging.

**New insights and molecular markers of each spirit have been found and hypotheses were made about the causes of their preponderance. The discussion could be further developed in a longer presentation.**

## IV.P.16

## PHENOLIC CHARACTERIZATION OF WHITE AND RED HYBRIDS OBTAINED FROM MONASTRELL AND OTHER VARIETIES

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**Keywords:** hybrids, Monastrell, Phenolic compounds, wine

Grapevines and therefore their corresponding wines are affected by the impacts of future climate change in terms of quality. In the Mediterranean area of Southern Europe, where Monastrell is the major variety planted will suffer the consequences due to a decrease of rainfalls associate with an increase of temperature in this location. Different strategies must be used to try to solve this actual problem. One of these alternatives could consist in the application of breeding programs using hybrids obtained by crosses between Monastrell grapes and other premium varieties such as Cabernet Sauvignon or Syrah.

In winegrapes, the technological importance of phenolic compounds, mainly flavonoids, is well-known. They are responsible for the color of wines, especially anthocyanins (colored pigments responsible for the chromatic characteristics of red wines), tannins (responsible for the long-term stability of red wine color), and flavonols (compounds that may influence wine color through copigmentation). Moreover, they influence on other organoleptic properties such as astringency, bitterness, and body (Ruiz-García *et al.* 2012).

In this work we have carried out the study by high performance liquid chromatography (HPLC) of the phenolic profile of grape and wine samples of white and red hybrids obtained from crosses between the Monastrell variety and other varieties (Cabernet Sauvignon, Syrah, Tempranillo and Verdejo) during the 2017 campaign.

The results showed less phenolic content in white hybrids than their parental varieties in both grape and wine. This difference was increased in wine samples due to the elaboration process used, different to the vinification of red wines. Tannic concentrations obtained in grape skins, seeds and white hybrid wines were similar to those of their parental varieties, showing grape samples a higher concentration than those of wine and being specially interesting the concentration of skins tannins. On the other hand, they showed total stilbenes content similar to those of their parental varieties.

Regarding red hybrids, they presented anthocyanins concentrations higher than those of their parents in both grape and wine. Total flavonols concentrations in grapes were similar to those shown by their parental varieties, with hybrids showing higher content than them in wine. Regarding the results obtained for tannins, it was found that in skin, seed and wine, total concentration was higher than that found in their parents. Finally, stilbenes analysis showed different results depending on the inherited influence of the parents, showing some hybrids high concentrations of T-resveratrol, which gives them a great nutritional and commercial interest.

IV.P.17

WHITE AND RED CROSSES FROM MONASTRELL VARIETY. AROMATIC AND SENSORIAL CHARACTERIZATION OF THEIR WINES

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**Keywords:** Aromas, GC-SPME-MS, Hybrids, Volatile compounds

The current climate change requires studying new varieties adapted to the dry climate of southern of Spain, Murcia. Besides, it is a great opportunity to try to introduce new white varieties, which are minority in this geographical area.

Aromas are an important quality factor in the organoleptic characteristics of the wine. A semi-quantitative analysis was carried out in order to study the aroma composition of different white and red wines from crosses of Monastrell. On one hand, firstly, it was studied 9 red crosses from Monastrell (M) with Cabernet Sauvignon (C) (MC104, MC111, MC18, MC4, MC80, MC80, MC85, MC94 and MC98); and secondly 2 crosses from Monastrell with Syrah (S), (MS10 and MS34). On the other hand, it was determinate the aromatic composition of 12 white crosses from Monastrell with other varieties; such as Cabernet Sauvignon, Syrah, Tempranillo (T) and Verdejo (V), (MC10, MC180, MC9, MC69, MS30,MS33, MS82, MT103, MT19, MV11, MV67 and MV7).

Around of thirty compounds was analysed and grouped into four aromatic families (acids, alcohols, esters and terpenes/norisoprenoids). The results showed how MT103 and the crosses MC180, MC69 stand out for their great aromatic qualities as white wines, having a great quantity of esters, acids and alcohols aromas which gives notes of fresh and fruit wines. In addition, MC104, MC18, MS10 and MS34 were considerate the most aromatics red crosses. Highlight, the alcohols found in these crosses as 3-methyl-1-butanol giving aromatic complexity to the wines and  $\beta$ -phenyl-ethanol with floral aromas and rose notes. Furthermore, MS10 was found as the most varietal crossing. These crosses had good quantities of esters, giving to the wines great red fruit notes.

In short, the results showed how the white and red crosses could be an interesting approach for having new varieties well adapted to the climate change in this area, which could also be introduced in other zones where the effects of high temperatures are also noticeable.

## IV.P.18

**AROMA CHEMICAL CHARACTERIZATION OF VALPOLICELLA WINES AND INFLUENCE OF GRAPE WITHERING PROCESS****Davide SLAGHENAUF**

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Aroma is one of the most important sensorial attributes that define the quality of a wine and can therefore influence consumer choices. Valpolicella is a long-established wine region where various different red wines are produced, including Valpolicella Classico and Amarone. Previous studies from our laboratory indicated that the volatile composition of these wines can be affected by factors such as grape variety and geographical origin, wine aging, and grape post-harvest withering practices, the latter traditionally associated with Amarone production.

This study aims to characterize aroma chemical features of wines from Corvina and Corvinone grapes, the two main varieties of Valpolicella. SPE-GC-MS, SPME-GC-MS and SPE-GC-O analysis were applied for this purpose. Wines were from 5 different vineyards located in 2 different regions of Valpolicella, they were vinified with a standard protocol in small batches. GC-O analysis, showed differences between varieties in terms of presence of specific odor zone (OZ) or odor intensity by mean of modified frequency value (MF%). Corvina wines showed a prevalence of OZ linked to floral aromas, Corvinone wines show a prevalence of earthy and vegetal aromas. Volatile compounds analysis showed that Corvina wines were characterized by high level of terpenes for Corvina wines, whereas Corvinone by  $\beta$ -damascenone content.

The geographical origin significantly marked these wines. It was found that some OZ with fruity aroma were able to distinguish wines according to their geographical origin. The identification of the molecule responsible for these aromas are in progress. Among the compounds that permitted to discriminate samples provenance there were esters,  $\beta$ -damascenone, and benzenoids like vanillin, 2,6-dimethoxyphenol and benzaldehyde.

Investigations on the aromatic influence of post-harvest withering, carried out on Corvina grapes, indicated that wines from withered grapes were mostly characterized by flower and spicy notes. The compounds responsible for these aromas are under investigation, MS tentatively identification suggested the presence of terpenes. Wines from not withered grape were characterize by more intense fruity OZ associated to ethyl propanoate and ethyl isobutyrate. However, with ageing this difference disappear due to esters hydrolysis. Wines from withered grapes were characterized by significantly higher content of  $\beta$ -citronellol, 3-oxo- $\alpha$ -ionol and terpinen-4-ol.

IV.P.19

## A BIBLIOMETRIC STUDY TO UNRAVEL THE SCIENTIFIC RESEARCH IN GRAPE AND WINE PHENOLIC COMPOUNDS

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**Keywords:** Bibliometric analysis, Phenolic compounds, winegrowing, winemaking

A significant amount of the viticulture and oenology research is dedicated to the antioxidant nature and the organoleptic role played by phenolic compounds. Despite the effort and the large amount of published information, the understanding of the phenolic compounds chemical features and the role played by these relevant group of compounds on the wine quality still remains inconclusive. A critical evaluation of the scientific research that is currently published on the topic could be obtained through bibliometric analysis. Quantitative methods are applied to evaluate measurable outcomes of research activity.

The information is obtained from research papers published in scientific journals contained in databases. The Web of Science Core Collection (WOS) database from Clarivate Analytics was used to perform a bibliometric study to evaluate the scientific structure in the phenolic compounds topic. However, the bibliometric search was restricted to provide an exclusive approach to grape and wine phenolics from a pure winegrowing and winemaking perspective. The results showed that a considerable amount of work was dedicated to understand the organoleptic role played by phenolic compounds. Secondly, despite the large body of knowledge found for the role these compounds play on the astringency and bitterness, it is foreseen that intense research efforts will be dedicated towards a more complete understanding of this phenomena. Finally, based on the results observed it can be theorized that the future research endeavours will focus on the comprehension of wine flavour compounds, in which phenolic compounds are expected to play an important role.

## A NOVEL OZONATED WATER APPLICATION STRATEGY IN BOBAL GRAPEVINES AND ITS EFFECT ON GRAPE QUALITY

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**Keywords:** ozonated water, endotherapy, spraying, Bobal

In viticulture, the use of chemical pesticides has become unpopular in recent years because of their negative impact on human health and the environment. In this context, ozone in aqueous solution is emerging as a new tool to control grapevine diseases mainly because it is considered environment-friendly and has broad-spectrum antimicrobial properties. However, little is known about the effect of these ozone treatments on grape quality.

The aim of this study was to evaluate the effect on grape quality of ozonated water treatments carried out in Bobal grapevines grown in Castilla-La Mancha (South-East of Spain). Two different application strategies were used: endotherapy (E), i.e. direct injection of the ozonated water into the trunk, as well as the combination of endotherapy and spraying (E+S). Ozonated water was applied through endotherapy four times before harvest for both E and E+S treatments. In the case of E+S treatment, spraying was done eight times before harvest, specifically a few days before and after each endotherapy application.

Grape quality on harvest day was evaluated through several enological parameters associated to the technological maturity, chromatic parameters such as colour intensity and tonality, the phenolic maturity and the Varietal Aromatic Potential Index (IPAv). The phenolic and volatile composition of grapes was also determined by HPLC-DAD and HS-SBSE-GC-MS, respectively.

Neither of the treatments significantly affected the technological maturity of grapes but both increased the total anthocyanin content at pH 1. In general, grapes treated by endotherapy showed greater phenolic maturity and content of phenolic compounds, while the combination treatment had a negative effect on these parameters. In terms of grape aroma, both treatments reduced IPAv but increased the content of free volatile compounds. These results suggest that ozonated water does not only improve the health of grapevines but also affects the quality of grapes; however, the effect on grape quality strongly depends on the application strategy.

IV.P.21

ACYLATED DERIVATIVES OF FLAVONOL 3-O-GLUCOSIDES IN VITIS VINIFERA AND HYBRID GRAPES GENOTYPES

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**Keywords:** phenolic compounds, flavonols, acylated derivatives, grapes

Flavonols are phenolic compounds derived from plant secondary metabolism and possibly the most diverse of the non-polymeric flavonoids in nature. Flavonols are predominantly synthesized in the grape skin where they are involved in UV screening. Moreover, these compounds play an important role on wine quality due to its effect on color stabilization through copigmentation and their antioxidant activity, especially in white wines.

In this work, we report the presence of acylated flavonols in red *Vitis vinifera* grapes (Tempranillo, Moribel and Tinto Fragoso) and, for the first time, to the best of our knowledge, in hybrid grape genotypes (BRS Vitoria and BRS Carmem) using HPLC-DAD-ESI-MS/MS. *p*-Coumaroyl derivatives of the methoxylated flavonol 3-*O*-glucosides were identified based on their UV-Vis and MS/MS spectra. In addition, to confirm the presence of these flavonols in grape samples, the study of the compound accurate mass was measured using HPLC-ESI-Q-ToF system.

Syringetin 3-*O*-(6"-*p*-coumaroyl)-glucoside was found in the *Vitis vinifera* and hybrid grape genotypes. However, the occurrence of *p*-coumaroylated derivatives of the flavonol 3-*O*-glucosides from the methoxylated aglycones isorhamnetin and laricitrin were only detected in BRS Vitoria grapes, compounds that were also reported for Tannat, Marselan and Syrah *Vitis vinifera* grape cultivars and their respective wines. In addition to the measurement of the accurate mass, the UV-Vis obtained of each *p*-coumaroyl derivative of flavonol closely matched the sum of the respective spectra of the flavonol 3-*O*-glucoside and that of *p*-coumaric acid. The results obtained suggest high variability in the flavonols profile of different grape cultivars.

## IV.P.22

## ANALYSIS OF POLYPHENOLS IN OVERRIPPED BOTRYTIZED GRAPES : SELECTIVE INDUCTION OF STILBENE OLIGOMERS

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**Keywords:** Stilbenes, Isohopeaphenol, *Botrytis cinerea*, Anthocyanins

The fungal pathogen *Botrytis cinerae* is a necrotrophic ascomycete responsible for the gray mold disease in grape. Mature and overripe berries are especially sensitive towards this pathogen under specific climate conditions of high humidity. Upon infection, there is an induction of metabolomic, transcriptomic and physiological changes. Genes involved in the polyphenols biosynthesis such as phenylalanine ammonia-lyase and stilbene synthase are upregulated in infected berries. Stilbenes are phytoalexins that are known to be key molecules for the resistance of grapevine against fungal diseases and studies in white noble rot botrytized grapes show that they can accumulate during infection.

The aim of our study was to compare the phenolic composition (anthocyanins and stilbenes) in infected and healthy berries of a red cultivar, Cabernet sauvignon, at the late stages of infection. To do so, three 1kg clusters each of botrytized and non botrytized grapes were collected in Bordeaux vineyards at the end of the season. 50 grapes of each cluster were manually peeled and the skins were frozen at -80, pulverized with a mortar and pestle and freeze dried. The polyphenols were extracted with acidified methanol (0.1% formic acid). Total polyphenols were measured by the Folin-Ciocalteu method. Individual anthocyanins were analyzed by HPLC-DAD while stilbenes were first identified by LC-Ion Trap and then quantified by LC-QqQ.

As expected because of the late period of collecting the grapes, the losses on total polyphenols were high (72%). All individual anthocyanins were highly reduced in infected grapes. Concerning stilbenes, there was a significant reduction in the contents of trans-resveratrol (50% losses in infected grapes), while there was a stimulation in the contents of total stilbene oligomers. Interestingly, our results show that not all the oligomers were stimulated: there were no significant differences for  $\epsilon$ -viniferin,  $\omega$ -viniferin, parthenocissin or hopeaphenol between the botrytized and non botrytized grapes. Major induction was found for pallidol (increases over 50%),  $\delta$ -viniferin (increase of 40%) and specially a stilbene tetramer, isohopeaphenol, which was stimulated over 10 fold in botrytized grapes.

IV.P.23

ANALYSIS OF STILBENES OF GRAPE BERRIES FROM WILD *VITIS* SPECIES BY LC-MS-QQQ

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**Keywords:** stilbenes, grape berries, wild *Vitis*, mass spectrometry

Nowadays, the strongest potential source of genetic resistance to biotic or abiotic stress for breeding programs in grape berries (*Vitis vinifera*) are the wild *Vitis* species such as American and Asian species. Stilbenes, which are phenolic compounds derived from the phenylpropanoid pathway of secondary plant metabolism, are known for their role as phytoalexins in Vitaceae family. Indeed, oligomeric forms of stilbenes are potent antifungal, oomycide and insecticide compounds. However, the presence of complex stilbenes in grape berries in different wild *Vitis* remains unexplored. To fill the gap and understanding the potential benefit of stilbenes in American and Asian species, the aim of the present work was to identify and quantify stilbenes in berry skin of several native *Vitis* species. The development of mass spectrometry methodology using triple quadrupole instrument and purified standards was conducted. Sixteen stilbenes were identified and quantified in thirteen wild *Vitis* species. The main stilbenes in grape berry skin were the monomers *E*-piceid, *Z*-piceid, *E*-isorhapontin, *E*-astringin, the dimers *E*- $\epsilon$ -viniferin, *Z*- $\epsilon$ -viniferin, pallidol as well as more complex stilbenes as the trimer *E*-miyabenol C and the tetramer isohopeaphenol. In addition, some tetrameric stilbenes such as vitisin A and vitisin B were characterized for the first time in grape berries. Focusing on wild *Vitis* species, the American species such as *V. champinii*, *V. rupestris*, *V. palmata* and *V. candicans* together with the Asian species *V. piazeskii* were the richest in stilbenes with values comprised between 80 and 300  $\mu\text{g/g}$  of dry berry skin. These findings could provide useful information for future breeding programs in order to enhance fruit quality and resistance in grapes.

## IV.P.24

## ANTIOXIDANT ACTIVITY AND POLYPHENOLS CONTENT OF GRAPES IN BELARUS

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**Keywords:** grape hybrid, ABTS, FRAP, total polyphenols

Grapes are a rich source of antioxidants. The antioxidant activity of grapes is positively correlated with the concentration of polyphenolic compounds. Polyphenols in grape determine not only nutritional value, but physical, chemical and organoleptic properties of wine and juice. The concentration of phenolic in grape berries depends on various genetic, environmental and cultural factors.

The study focused on antioxidant activity and total phenol content in grape berries from four hybrids cultivars (*Vitis vinifera* × *Vitis riparia*): V-1, V-2 (red grapes) and V-3, V-4 (white grapes). Grapes are cultivated on the Ecology Center of Brest State A.S. Pushkin University (Brest, Belarus). They are varieties for universal using. These hybrids of grape have a good degree of maturation of the vine, and characterized by high cold and disease resistance.

Antioxidant activity of grape berries was determinate using ABTS radical scavenging capacity and ferric reducing antioxidant potential (FRAP) assays. Antioxidant activity values increased from 8.5 to 29.6 μmol Trolox equivalent/100 g of fresh weight (FW) for ABTS and ranged from 2.72 to 39.2 μmol Fe<sup>2+</sup>/100 g FW for FRAP method.

Two methods identified the same order of reducing the antioxidant activity: V-2 ≈ V-4 > V-1 > V-3.

Total polyphenolic content was determined by the Folin-Ciocalteu method and the results were expressed in mg gallic acid equivalent (GAE). This parameter ranged from 182.88 (for white grapes) to 399.37 (for red grapes) mg GAE/100 g FW and reduced in order: V-1 > V-2 > V-4 > V-3. The difference in polyphenol content between the studied cultivars was significant.

Thus, the polyphenolic compounds are the basic contributors to the antioxidant properties of grapes and depend on cultivar.

#### IV.P.25

### ANTIOXIDANT CAPACITY AND STILBENOID LEVELS IN GRAPE CANES: EVOLUTION DURING POST-PRUNING STORAGE

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**Keywords:** Antioxidant capacity, Grape cane , Post-pruning storage, Stilbenoids

*Vitis vinifera* canes properly handled during post-pruning storage can be a relevant source of stilbenoids, mainly E-resveratrol and E- $\epsilon$ -viniferin [1-3]. De novo E-resveratrol biosynthesis in grape canes is triggered by stress conditions. Its levels depend on storage conditions (temperature, time, length of cane pieces and relative humidity [1-4]. Suitable conditions for canes to enhance stilbenoid levels, mainly E-resveratrol, is storage during 3-4 months, at 15-20°C and 60% of relative moisture [1-3]. If canes are cut in smaller pieces, it is possible to reduce the post-pruning time to enhance the stilbenoid levels [4]. In humans, stilbenoids are associated to defense against aging-related diseases, suppressing the oxidative stress effects due their putative antioxidant capacity [5]. Isolated stilbenoids from grape canes have shown antioxidant capacity by different In vitro assays [6-7]. Oligostilbenoids as miyabenol C, pallidol, scirpusin A, ampelopsin A and E- $\epsilon$ -viniferin have a higher antioxidant capacity by ORAC-FL assay than E-resveratrol [6].

The present work is focused on the evolution of antioxidant capacity and stilbenoids during post-pruning storage in canes of four red varieties cultivated in the Itata valley in Chile (Cabernet Sauvignon, Pinot Noir, Tintorera, Cinsault, Malbec). 20 cm long cane pieces were stored during 90 days after pruning at 20°C and 60% relative humidity in darkness. Stilbenoids and catechins were monitored by HPLC-UV and antioxidant capacity by ORAC-FL, ABTS and CUPRAC assays. The highest antioxidant activity was observed for Pinot Noir and Cabernet Sauvignon canes (450 - 1300  $\mu\text{mol/g}$ ) by ORAC-FL assay. Pinot Noir, Tintorera and Malbec reached the highest stilbenoid concentration (7.552 - 5.498 mg/kg). Surprisingly, evolution of antioxidant capacity in canes does not show a significant increase during storage, as it was observed for E-resveratrol. Further investigations are required to understand better the unexpected changes of antioxidant capacity during post-pruning storage.

*Acknowledgements:* FONDECYT 1150721 and AFB170007 grants from CONICYT- Chile.

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## IV.P.26

## AROMATIC CHARACTERIZATION AND EFFECT OF AGING IN LUGANA, PINOT GRIGIO AND VERDICCHIO WINES

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**Keywords:** White wines, Aging, Sensorial analysis, Gas chromatography – mass spectrometry (GC-MS)

Lugana, Pinot grigio and Verdicchio are white wines representative of long established Italian winemaking regions. Lugana and Verdicchio are derived from the same grape variety but are produced in the regions of Veneto-Lombardia and Marche respectively. Pinot grigio is produced in the regions of Veneto, Friuli and Trentino. In spite of their commercial relevance for the respective production areas, there is little knowledge concerning their aroma characteristics. Moreover, especially in the case of Lugana and Verdicchio, there is a considerable interest towards production of wines for longer aging (Riserva).

In this work we investigated the aroma composition of commercial Lugana, Pinot grigio and Verdicchio wines in order to gain insights in the main aroma compounds of each wine type. The effect of accelerated aging on these aroma compounds was also investigated. For both young and aged wines, sensory evaluation was also carried out. Young Lugana wines were characterized by higher content of terpenes, benzenoids, and methyl salicylate, which could be a marker of this wine. Verdicchio wines had relatively similar volatile composition, although terpene alcohols and methyl salicylate levels were lower than in Lugana. These observations are in agreement with the fact that these wines are from the same grape variety. As for Pinot grigio wines, they were mostly characterized by fermentation-derived aroma compounds. Following aging, Lugana and Verdicchio wines appeared mostly characterized by various cyclic terpenes including p-cymene and 1,4-cineol, the latter at concentrations clearly higher than the reported odor threshold. Lugana still displayed high levels of methyl salicylate after aging. With regard to Pinot grigio, aging induced accumulation of DMS at concentration greater than Verdicchio and Lugana, although at relatively low levels compared to existing literature.

At sensory level, the wines were described with attributes such as tropical fruit, floral and yellow fruit. After the aging, they were described like, herbaceous, fruit, floral and rusty. The oxidation and solvent notes increased with aging. Although in young wines a reasonably good classification of the samples according to wine type was obtained, in aged wines the classification did not follow a pattern by varieties. The knowledge of these key volatile compounds could assist in optimizing winemakers decision concerning aroma styles and longevity of these wines.

IV.P.27

AROMATIC POTENTIAL OF MERLOT AND CABERNET SAUVIGNON BERRIES: IDENTIFICATION AND ASSAY OF GAMMA-NONALACTONE PRECURSORS

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**Keywords:** flavors, lactone,  $\gamma$ -nonalactone, precursors

Wine flavor results on complexes interactions of odorous components, which come from different aromatic families like esters, thiols, aldehydes, pyrazines or lactones<sup>1</sup>. Lactones contribute to aromas reminiscent of stone fruits such as peach or apricot, and often evoke coconut in red and sweet white wines<sup>2</sup>. Recent studies have demonstrated the key role of lactones in red wine. For example,  $\gamma$ -nonalactone, responsible for cooked peach flavors, was detected in must and found at higher concentrations in wine (Beyond its detection threshold, Dth 27  $\mu\text{g/L}$ )<sup>3</sup>. Those observations tend to show that  $\gamma$ -nonalactone could be produced during alcoholic fermentation as observed in beer<sup>4</sup>.

In this way, this project aim at identifying  $\gamma$ -nonalactone precursors in berries, and wine in order to investigate their metabolic pathways. We present the first results related to the assay of precursors of this lactone in must as well as the impact of alcoholic fermentation on the formation of  $\gamma$ -nonalactone in wine.

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## IV.P.28

## AUSTRIAN PINOT BLANC WINES: TYPICITY AND WINE STYLES

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**Keywords:** GC-SIM-MS, volatile profile, sensory study, Pinot blanc styles

With a total cultivated area of 1,914 hectares, Austria is the third largest Pinot blanc-producing country in the world (15,493 hectares). Typical Austrian Pinot blanc wines reveal aromas described with pear, apple, quince, banana, apricot, caramel, and citrus fruits. Mature wines are reminiscent of fresh bread and gain in density and structure through cask storage and longer bottle ageing. The Pinot blanc lacks aroma intensity, but its aromas are perfectly expressed in young wines. Pinot Blanc can be found in all wine regions of Austria. However, it plays in comparison to the most significant white wine variety Grüner Veltliner an economically subordinate role. One of the main reasons for this is that, with the exception of the Leithaberg DAC, there are no defined wine styles for wines of this grape variety in Austria.

The current study aimed to uncover different common styles of Austrian Pinot blanc wines and evaluate the differences and similarities analytically and sensory. Six different valid GC-SIM-MS methods with different sample preparation systems (head-space-solid-phase-micro-extraction, solid-phase-extraction, liquid-liquid-micro-extraction) were developed for the quantification of 108 different volatile compounds and in total more than 150 Austrian Pinot blanc wines of the vintage 2015, 2016 and 2017 were analysed. A trained panel, an expert panel and a consumers panel evaluated these 150 wines on their typicality.

Using different sensory studies and statistical tools, it was found out that there was a clear separation between four different Pinot blanc wine styles within these wines. These four groups were briefly described with the terms citrus (1<sup>st</sup> style), green apple and green pear (2<sup>nd</sup> style), ripe yellow apple, quince, cooked pear, bread and honey notes (3<sup>rd</sup> style), as well as intense oak notes (4<sup>th</sup> style).

A Kruskal Wallis test revealed that there was a significant difference ( $p < 0,05$ ) between these different styles in content of some esters, higher alcohols and volatile phenols. Future work will focus on the contribution of these significant volatile compounds to the Pinot Blanc flavor.

IV.P.29

**AUTHENTICITY CONTROL OF BEVERAGES AND WATER WITH GASBENCH II SYSTEM USING HYDROGEN AND OXYGEN ISOTOPE FINGERPRINTS**

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**Keywords:** Wine, Isotope Analysis , Food Authenticity, IRMS

In this presentation the application of **stable isotope fingerprints in beverage and water in food** is explored. Data are shown that show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints in beverages and the technology used is also provided. It can easily be adapted to water in food (e.g. meat).

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect whether the labeled product is authentic or if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.

Detecting food and beverage fraud can be achieved using stable isotope measurements with the isotope equilibration technique (**Method OIV-MA-AS2-12, EU regulation no. 822/97**). This because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable standardized technique in food and beverage fraud prevention and food safety. For wine in the EU isotope signals are stored in the **EU-WineDatabase** or other **national databases**, e.g.  $>1400 \delta O18$  of water in European wines, national databases).

**IV.P.30****AUTOMATED TARTARIC ACID ANALYSIS IN WINE USING A DISCRETE ANALYZER****Liisa OTAMA**

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**Keywords:** Tartaric Acid, Discrete analysis, Gallery Analyzer, Automated discrete analyzer

Acids in the right combination provide wine with their crisp, slightly tart taste. If a wine is too low in acid, it tastes flat and dull; too high in acid and it is tart and sour. Tartaric acid, not found in most other fruit, is the primary acid in the grape and thus controls the acidity of a wine. It plays a critical role in the taste, feel, and color of wine. More importantly, it lowers the pH to a level that improves resistance to bacterial contamination, acting as a preservative. Tartaric acid deficiency therefore, can contribute to various wine issues.

The purpose of this study was to evaluate the determination of tartaric acid in red wines using Thermo Scientific™ Gallery™ automated discrete analyzer. Tartaric acid method is based on formation of a complex between tartrate and vanadate. There is no need for pretreatment of red wine samples since the red color is removed by hypochlorite during the automated procedure.

The evaluated red wine method correlated well ( $y = 0.9627x + 0.235$ ,  $R^2 = 0.9304$ ) with flow injection analysis (FIA) method.

IV.P.31

## CHANGES IN SKIN FLAVANOL COMPOSITION AS RESPONSE TO OZONE-INDUCED STRESS DURING POSTHARVEST DEHYDRATION OF RED WINEGRAPES WITH DIFFERENT PHENOLIC PROFILE

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**Keywords:** Ozone exposure, Dehydration process, Flavanols, Postharvest winegrapes

Secondary metabolism of winegrapes remains active after harvest and the osmotic stress associated with the dehydration process can induce the accumulation and/or degradation of phenolic compounds. Regarding skin flavanols, the concentration of monomeric and oligomeric compounds usually decreases when grape dehydration progresses, leading to an increased mean degree of polymerization (mDP). In addition, biosynthetic pathways can be induced in winegrapes undergoing oxidative stress during ozone treatment as a consequence of the chemical response against tissue oxidative damage. Nowadays, the combined effect of abiotic stresses is being used to promote controlled overproduction of target secondary metabolites.

In this study, the combined effect of postharvest dehydration and long-term ozone treatment of red winegrapes was evaluated as a strategy to induce compositional changes in skin flavanols. The experiment was performed in thermohygro-metric controlled chambers at 20 °C and 70% relative humidity with a continuous gaseous ozone concentration of 30 µL/L. Grape berries were sampled at 10% and 20% weight loss. This dehydration process was compared with a separate trial under air atmosphere at the same environmental conditions. The two red winegrape varieties studied were Barbera and Nebbiolo (*Vitis vinifera* L.) for their different phenolic composition and cell wall characteristics. The flavanol composition of berry skins was determined by size exclusion chromatography and reversed-phase HPLC after phloroglucinolysis.

The results obtained showed that the ozone effect on skin flavanol profile and concentration was variety dependent. In Barbera berry skins, characterized by relatively low proanthocyanidin concentrations with low polymerization degree, the higher molecular mass fractions of flavanols slightly decreased during dehydration under air and ozone-enriched atmosphere, and the lowest value of mDP corresponded to fresh samples due to their richness in medium molecular mass flavanols. Ozone treatment led to an increase in medium-high and medium molecular mass flavanols when compared to air-exposure for samples dehydrated up to 10 and 20% of weight loss. Nebbiolo skins are characterized by a higher fraction of highly polymerized flavanols particularly in fresh grapes, whose greater decrease corresponded to grapes dehydrated at 10% weight loss under ozone-enriched atmosphere, probably due to partial de-polymerization that was not significant for the mDP value.

## IV.P.32

**CHARACTERIZATION AND CYTOPROTECTIVE EFFECTS OF POLYPHENOLIC EXTRACTS OF WINES ON NEURONAL CELLS****Grégory DA COSTA**

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**Keywords:** wine extracts, polyphenols, cytoprotective effects

**Background.** Numerous studies have shown that the Mediterranean diet can be beneficial for health. This diet includes fruit, vegetables, olive oil, fish and a moderate consumption of wine. With regards to wine consumption, these beneficial effects can be linked to richness in varieties and quantities of the phenolic compounds present in wines. Based on this observation, we studied the chemical composition and the cytoprotective activities of extracts of different grape beverages (white wine, rosé wine, red wines and grape juice).

**Methods.** Isolated phenolic extracts were characterized by NMR and MS. The cytoprotective activities of total phenolic extracts of four wines (Tannat red wine, Merlot red wine, Merlot rosé wine, Blanc Sauvignon wine) and a grape juice were studied by evaluate their ability to reduce  $\beta$ -amyloid induced toxicity on PC12 cells. The extract was subjected to a fractionation and the cytoprotective activities of the fractions were carried out. The concentrations of total phenolic compounds in these extracts and fractions were determined by colorimetric assay. The inhibitory potential of extracts with respect to the aggregation of the  $\beta$ -amyloid peptide was determined by fluorescence, and the mechanism of action studied by proton NMR.

**Results.** Three total extracts (two of red wines and one of grape juice) have cytoprotective activities. The polar compounds (organic phase) and the medium and low polar compounds (aqueous phase) were separated by fractionation. Fractionation revealed that the phenolic compounds present in the aqueous phases, mainly anthocyanins, are responsible for the biological activities of the total extracts. The cytoprotective effects were due in part to the ability of polyphenols to inhibit the aggregation of  $\beta$ -amyloid observed by fluorescence and NMR. Extracts from Tannat wines reduce the aggregation of the  $\beta$ -amyloid peptide by 80% by interacting strongly with this peptide.

**Conclusion.** This study provided evidence that anthocyanins were responsible for the biological activity of aqueous phases. The interaction between polyphenols from wine extracts and  $\beta$ -amyloid would reduce the formation of  $\beta$ -amyloid oligomers toxic to neuronal cells.

#### IV.P.33

### CHARACTERIZATION OF ANTHOCYANINS OBTAINED FROM CABERNET SAUVIGNON AND MERLOT GRAPE SKINS

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**Keywords:** anthocyanidins, CPC, grape skins

Anthocyanins are colored phenolic compound that are released from grape skins into the must during the winemaking process. They are responsible of the colour of red wine.

In the last few years, the sensory properties of anthocyanins are being reconsidered and several studies point out that they could be involved in the development of several oral sensations such as astringency or bitterness.

It is well established that the optimal maturity level is related to the obtaining of high quality wines, nevertheless, the climatic change risks to change the current scenario and increase the lag between technical and phenolic maturity.

In this scenario, the loss of wine tipicity is one of the risks to be expected, primarily in Bordeaux region, which is classified as a high risk area for climatic change impact.

Therefore, there is an interest in increasing the knowledge of the role of anthocyanins in the development of oral sensations.

In this study, Merlot and Cabernet sauvignon grapes were selected since they are the most abundant ones in the Bordeaux region.

The isolation of the compound is carried out using CPC (centrifugal partition chromatography). Several mixtures have been tested and different fractions have been collected.

The compounds in each fraction are identified using an HPLC-DAD-MS. Fractions with the same composition are gathered together prior to characterization essays.

For the characterization essays, different pH and ethanol content are tested in order to find a correlation between the sensations elicited by the compounds and the medium characteristics.

## IV.P.34

## CHARACTERIZATION OF SAUVIGNON BLANC MUST FROM DIFFERENT ORIGINS

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**Keywords:** Chemical Analysis, Sauvignon Blanc

Wine of the same cultivar can have different characteristics when grown in different regions. This is due to different climatic conditions during the ripening process, harvest dates and different fermentation and storage conditions. In this work Sauvignon Blanc must from various origins in Styria, Austria and three different locations in Croatia, with harvest times from late August to October is compared using different analytical methods. Austria and Croatia both belong to the cool climate viticulture. Sauvignon Blanc from these regions usually exhibits green characteristics. One of the main aroma compounds responsible for this aroma is 2-Isobutyl-3-methoxy-pyrazine (IBMP), (Parr *et al.* 2007) It is responsible for the characteristic green bell pepper notes in cool climate Sauvignon Blanc wines as its concentration greatly depends on the climatic conditions during the ripening phase of the grapes and the harvest date. With a sensory threshold of about 1 ng/L (Allen and Lacey 1991) it influences the aroma even in low concentrations. The compound is present in the grape must in its free, odour active form.

For the analysis of the volatile fraction of the must, gas chromatography (GC) coupled with different mass-sensitive detection (MS) methods is used. For the quantification of important aroma compounds, like IBMP, GC with tandem MS is used. Additionally, results from several standard chemical wine analysis methods, like sugar content and titratable acidity, indicating ripeness of the grapes and the harvest dates will be taken into account. The results are compared using principal component analysis, visualizing differences and similarities between the samples.

The analysis of the same cultivar from different origins can give insight into the effect the different conditions have on the raw material. A characterization of the must can help when making predictions about the finished wine and choosing appropriate viticultural practices and further vinification methods to fit the raw material from different growing conditions.

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IV.P.35

COMPARISON OF BASIC CHEMICAL PARAMETERS AND PHENOLIC COMPOUND PROFILES IN WINES OF DIFFERENT CABERNET SAUVIGNON SELECTIONS IN THE AREA OF CAUQUENES, CHILE

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**Keywords:** Cabernet Sauvignon, Clonal and mass selections, Wine components, Phenolic compounds profiles

Cabernet Sauvignon is the most renowned and one of the most planted grape cultivars in the world to produce fine red wines. Chile is the second country, after France, with the highest national surface planted with Cabernet Sauvignon (42.409 ha). Indeed, Chile has positioned itself in the world as a producer of high-quality red wines and Cabernet Sauvignon is with 30,9 % of the vineyard surface, the principal grape variety planted for wine production. In the last decade raised up the interest to evaluate clonal and massal selections of Cabernet Sauvignon to expand the areas dedicated to production of high quality wines based on this cultivar.

In this context, besides the evaluation of viticultural and productive parameters of this plant materials in the area of Cauquenes, the specific aim of present work was to compare the basic chemical parameters and the profiles of phenolic compounds determined by HPLC-DAD (anthocyanins, catechins, flavonols, hydroxycinnamic acids) in experimental wines made with grapes of a massal selection of Viña Concha y Toro and with three INRA-ENTAV clones: 169, 191 and 337 during five successive vintages at the same location near Cauquenes.

Regarding basic wine physicochemical parameters, minor differences were observed in the alcohol content (13,8 -14,1°), total acidity (3.70-3,92 g L<sup>-1</sup>), volatile acidity (0,35-0,40 g L<sup>-1</sup>) and pH (3,41-3,45). The wine made from the massal selection had the highest color and total polyphenol index, whereas the clone 169 showed the lowest values for those two parameters. Tannins were also higher for the massal selection, and lower for the clone 169, as well as for the clone 337, while the clone 191 had a medium level.

The wine of clone 191 appeared as the plant selection with the highest level of total anthocyanins and all monomeric anthocyanins determined by HPLC, followed by the clone 337. The same was observed for total flavanols and for hydroxycinnamic acids, where it showed superior values compared to the other clonal selections 337 and 169. The massal selection had higher values of catechin and epicatechin, followed by the clone 191.

These results let conclude that under the conditions of Cauquenes, in central-south Chile, different Cabernet Sauvignon selections have an incidence on levels and profiles of phenolic compounds, such as anthocyanins, flavonols, hydroxycinnamic acids, catechins and methylcellulose precipitable tannins.

*Acknowledgements: PI-599/CII-001*

## IV.P.36

## CORKLINS IN WINE

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**Keywords:** Cork, wine, polyphenols, catechin

Cork stoppers are traditionally employed in wine bottles to seal and protect the wine allowing at the same time for a proper wine ageing [1,2]. Different polyphenols have been found to migrate from different cork stoppers into bottled wine model solutions [3], being ellagitannins the most relevant ones. The aim of this study was to identify and characterize new ellagitannin-derived compounds in cork and to evaluate the reactivity of these compounds in model solutions with a major wine component, (+)-catechin. This reactivity yields to the formation and identification of several ellagitannin-catechin-derived compounds with a more complex structure. These newly-formed compounds may have an impact on the resulting wine sensorial properties. One of these new classes of ellagitannin-catechin-derived compounds are the corklins [4]. Furthermore, the work has been evolving towards the detection of corklins in commercial wines and may eventually be used as cork wine markers.

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The authors thank to PORTUGAL 2020 program – CorkPlus Project for the financial support and UID/QUI/50006/2013 – POCI/01/0145/FERDER/007265. This work was supported by FCT (SFRH/BPD/112465/2015) and (SFRH/BD/139709/2018) and an investigator contract (IF/00225/2015)

IV.P.37

## DETERMINATION OF ELLAGITANNINS IN WINE AGED IN BARRELS WITH DIFFERENT OXYGEN TRANSFER RATE

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**Keywords:** oxygen, ellagitannins, OTR, French oak wood

The ellagitannins present in oak wood are hydrolyzable and oxidizable tannins, which during the aging process of wines in barrels are involved in reactions with other wine compounds, affecting wine sensory characteristics. The levels of these compounds in oak wood are 10% of the dry weight and vary depending on the species, origin, treatment, etc. Oxygen is also an important factor in wine barrel aging, so it is important to know the oxygen transfer rate (OTR) provided by each barrel to the wine. The OTR depends on the structure of the wood and is related to the composition of the wood, and as a consequence with its content in ellagitannins.

The aim of this work has been, on the one hand, to evaluate the content in ellagitannins of the wood of the barrels of different OTR and, on the other hand, to study the evolution of their content in a red wine aged in those barrels of different OTR. Ten French oak barrels, *Quercus petraea*, were built at Intona SA (Navarra, Spain). For this purpose, the oak wood rough staves were classified according to their OTR for the construction of 4 barrels with high oxygenation potential and 4 barrels with low OTR. In addition, 2 barrels of the same oak were studied, made without wood classified by its OTR and which are the control barrels.

The concentration of the main four oak ellagitannins (castalagin, vescalagin, grandinin and Roburin E) was analysed in the oak wood extracted from the residues of each of the 10 barrels. On the other hand, a red wine from the DO Ribera del Duero (Spain) was aged in barrels for one year, being sampled at 6 and 12 months to evaluate the evolution of the content in ellagitannins. Analyses were performed by HPLC-DAD-MS using galocatechin as internal standard, after a two-step fractionation method consisting of a solid phase extraction (SPE) with C-18 cartridges followed by size exclusion chromatography in hand-packed Sephadex LH-20 columns, in the case of wine samples.

The results show that the content in ellagitannins is significantly lower in the wood of high oxygenation barrels compared to the wood of low oxygenation barrels. This difference is reflected in the red wine they age, so it has been found that wines aged in low oxygenation barrels have a higher content in ellagitannins than wines in high oxygenation barrels.

*Funding: This work was financed by the Interreg España-Portugal Programme (Iberphenol) FEDER and MINECO (AGL2014-54602P)*

## IV.P.38

**VARIETAL DIFFERENTIATION OF CORVINA AND CORVINONE YOUNG WINES BASED ON TERPENE PROFILES****Tiziana LISANTI**Maria Tiziana Lisanti <sup>1</sup>, Giovanni Luzzini <sup>2</sup>, Davide Slaghenaufi <sup>2</sup>, Stéphanie Marchand <sup>3</sup>, Gilles de Revel <sup>3</sup>, Luigi Moio <sup>1</sup>, Maurizio Ugliano <sup>2</sup>

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**Keywords:** Corvina, Corvinone, Soils, Mint aromas

Terpenes are one of the most important chemical classes among wine aroma compounds. Their biosynthesis in grapes is under genetic control, although environmental conditions modulate their expression.

Besides the aromatic grape varieties, which have a significantly higher terpene content, the so called “neutral varieties” may present very different terpenic compositions, in term of kind of compounds and concentrations. Terpenes are known to confer to wine floral and citrus aromas. However, very recently a small pool of limonene-derived terpenic compounds (i.e. piperitone, p-menthane lactones, carvone) were found to be responsible for the minty and fresh notes that play a key role in the ageing bouquet of red Bordeaux wines. While the biochemistry and the chemistry of the “floral” terpenes is quite well known, the origin and the evolution of minty terpenes in wine is still unknown. The hypotheses potentially explaining their “revelation” are numerous: existence of the compounds in a glycosidically-bound form or their precursors, formation during wine ageing, or unmasking of minty odorous notes due to changes in the volatile composition. The observation of a correlation between the percentage of Cabernet Sauvignon in the blend and the levels of piperitone in the old wines suggests that the concentration of these compounds may be variety-dependent. In the present study we defined the terpenic profile of two Italian grape varieties: Corvina and Corvinone. These varieties give origin to the prestigious Amarone wine, after partial drying and vinification.

Experimental wines were produced according to a standard protocol from Corvina and Corvinone grapes cultivated in five different terroirs. Most of compounds were determined by Solid Phase Extraction (SPE) and Gas Chromatography –Mass Spectrometry (GC-MS) analysis, while to determine the pool of minty terpenes, present at trace levels, it was necessary to carry out a double pre-concentration and extraction step, by coupling Solid Phase Extraction (SPE) and Stir Bar Sorptive Extraction (SBSE). Results revealed that Corvina wines were significantly richer in terpenic compounds, in all the considered terroirs.

As concerns minty compounds, 1,8-cineol, pulegon, carvon and piperiton were always more concentrated in Corvina wines, while Corvinone was richer in menthylacetate and mintlactone. Surprisingly the concentrations of mintlactone in young Corvina and Corvinone wines were in the range determined in long aged Bordeaux wines.

IV.P.39

## DEVELOPMENT OF A RAPID HPLC-TQ METHOD TO DETERMINE ELLAGITANNINS IN OAK WOOD AND COGNAC: EVOLUTION OF THEIR CONCENTRATION DURING COGNAC AGEING

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**Keywords:** ellagitannins, oak wood, Cognac, HPLC-TQ

Ellagitannins are the predominant phenolic compounds of oak wood (10% of dry weight). Vescalagin and castalagin are the monomers, they represent 50% of ellagitannins. Derivatives of these molecules are the dimers (roburin A, roburin D) as well as derivatives of xylose / lyxose (grandinin, roburin B, roburin C and roburin E). These compounds are responsible for high wood durability and they contribute to astringency sensation and bitterness taste. A lot of studies have been performed concerning wine quality (Chira *et al.*, 2012), however spirits quality has not been studied profoundly. More than 500 aromatic compounds have been detected in Cognac whereas polyphenols have not been studied profoundly. Particularly, studies on total polyphenols of brandies and American whiskeys were conducted, but their sub families have not been researched. Molecular ellagitannins evolution during Cognac ageing has never been studied.

The possibility that these molecules are submitted to transformations during aging in oak casks, which can last for several years should be taken into account. Consequently, spirits polyphenols represent a set of complex and unknown chemical structures. These structures, which are not only impacting the organoleptic quality of wine and spirits but also they possess antioxidant capacities (Canas, 2017), are not well known. Furthermore, the existing methods to characterize molecular ellagitannins are rather long and missing validation. Thus the aims of our study were to develop and validate a rapid method to identify and quantify Cognac and oak wood ellagitannins and observe their evolution during aging. In order to achieve the above objectives, oak wood in maturation and Cognac representing different ages were analyzed. Furthermore, the evolution of these molecules in model solutions of spirits was researched. For ellagitannins oak wood extraction, a 350 ASE Dionex Corporation Extractor was used with an acetone/water 70/30 solvent.

The evolution of ellagitannins in Cognac and oak wood was followed by an HPLC-TQ. The method was validated by studying sensitivity, linearity in working range, intraday repeatability, intraday precision, trueness and specificity.

**IV.P.40****DIFFERENCES IN XYLOVOLATILES COMPOSITION BETWEEN CHIPS AND BARREL AGED WINES.****Maurizio PETROZZIELLO**

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**Keywords:** Xylovolatiles, barrel, chips, GC-MS

This research was primarily focused at the optimization of a feasible GC-MS method for the characterization of xylovolatile aromatic compounds in wines in order to highlight any compositional differences between wines aged in barrels and those obtained using alternative products (chips). About 150 commercial barrel aged wines were analyzed. Moreover about 40 wines (red and white) were aged at the experimental winery of the Research Centre of Viticulture and Enology in Asti using 14 different types of commercial oak chips

Sample preparation for GC analysis was easily carried out by a single step by Solid Phase Extraction, using polymeric SPE cartridges with a high binding capacity and multiple retention mechanisms to retain the broadest spectrum of aromatic compounds. More than 60 volatile molecules from oak have been identified by GC-MS and semi-quantified using an appropriate internal standard. Analytical results were explained by multivariate statistical analysis. A preliminary step was carried out using principal component analysis (PCA), which showed interesting compositional differences between barrel and chips aged wines namely methyl and ethyl vanillate as well as furan derivative compounds. Afterwards, in order to test if selected chemical explanatory variables allow to discriminate treatments and how they are useful to predict which group a new observation will belong to, a discriminant analysis (DA) was carried out. Several wine groups were considered, defined by factors including oak wood toasting degree, wood geographical origin and the wine typologies (red and white).

Furthermore, Near Infrared Spectroscopy analyses were performed on a subset of 30 red wines, 15 of which refined with chips. This part of the work was aimed at identifying possible applications of NIR for discriminating wines treated or not with chips, by identifying possible spectral zones linked to xylovolatiles and related to any compositional differences. NIR results were compared to the GC-MS data and a close relation between transmittance at certain wavelengths and xilovolatiles were observed. Namely, spectral zones correlated to furanaldehydes derivatives appear to be the most informative to discriminate between oak and non-oak treated wines.

#### IV.P.41

### DIFFERENTIATION OF EUROPEAN PINOT BLANC WINES ACCORDING TO VOLATILE ORGANIC COMPOUNDS (VOCs) USING HEADSPACE-SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-TIME OF FLIGHT-MASS SPECTROMETRY (HS-SPME/GC-TOF-MS) ANALYSIS

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**Keywords:** VOCs, Pinot Blanc, PCA, SPME/GC-ToF-MS

Pinot blanc is a white wine characterized by a relatively high acidity and fresh-fruity aromas such as citrus, melon, pear and apricot with slightly smoked or mineral undertones. The pinot blanc cultivar originated from the Alsace region of France. Nowadays this variety is cultivated over various European wine regions including Austria, Germany and South Tyrol (Italy). The aroma, and more generally the volatile organic compounds (VOCs) are used for determining wine character, quality, and authenticity.

Wines VOCs profile is widely studied with HS-SPME/GC-ToF-MS. However, to date, there are only a few scientific papers dedicated to Pinot blanc VOCs investigating the relationship between site and the volatile composition of this wine. The aim of the present study is to differentiate Pinot blanc wines from different regions using their VOCs profiles.

A total of eight wines from Austria, Germany and South Tyrol were considered in this experiment. Samples were analysed using HS-SPME/GC-ToF-MS, and VOCs were identified using authentic standards.

Three replicates of each wine were measured in a randomised complete block design, and a QC sample (equal aliquots of each wine) was analysed every five samples to monitor technical variability. At the sampling time, the same wines were subjected to sensory analysis by a trained panel of wine consumers. A principal component analysis (PCA) was used to develop a discriminant model, and eight VOCs (including six alkyl esters, one primary alcohol and acetic acid) allowed the differentiation of three out of eight wines.

Moreover, ANOVA confirmed what revealed during the description of sensory impressions.

Particularly, wines with a roasted and smoked aroma were characterized by 2-methylbutan-1-ol, a primary alcohol responsible for roasted aroma. Conversely, wines that have been described as "sweet" and "floral" by the sensory analysis were primarily characterized by a high abundance of benzaldehyde and several alkyl esters. Moreover, PCA clearly discriminated both wines, indicating that there is a clear correlation between the sensory evaluation, the chemical analysis of VOCs, and the origin of the tested wines. In conclusion, the use of chemical and sensory data associated with PCA and ANOVA can provide a clearer differentiation of Pinot blanc wines belonging to several winegrowing areas.

*Acknowledgements: The "PinotBlanc" project has received funding from the European Regional Development Fund, ERDF 2014-2020.*

## IV.P.42

## DIFFERENTIATION OF SPANISH RED WINES BASED ON THEIR PROTECTED DESIGNATION OF ORIGIN AND CATEGORY USING PHYSICO-CHEMICAL PARAMETERS

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**Keywords:** Red wines category, Protected Designation of Origin, Physico-chemical parameters, Linear Discriminant Analysis

The protected designation of origin (PDO) recognition have an important role in the wine industry because of, in general, the consumers associate it with wines that have higher quality than others that not have this recognition. In addition, wine category (young or aged in oak barrel and bottle) is also an important factor that the consumers usually takes into account when buying a wine.

The aim of this study was to differentiate red wines from "Castilla y León" Spanish region by their PDO and wine category (young, "oak", "crianza", "reserve/gran reserve"). Hundred and thirty five wines were analysed from four PDO: Ribera del Duero, Bierzo, Toro and Cigales. Forty one physico-chemical parameters of red wines were analysed using spectrophotometric, HPLC-DAD, HPLC-RID and GC-FID analytical methods. Stepwise discriminant analysis is applied to find a linear combination of the variables that characterizes or separates two or more classes of objects. In this study, the forward method was used to select the variables most useful for differentiating the wines according to PDO or wine category.

LDA allowed a good separation between the wines from the different PDOs using 3 discriminant functions. The discriminant function 1 allowed a separation between the wines from Ribera del Duero and Bierzo and those from Toro and Cigales. On the other hand, the discriminant function 2 allowed separating, mainly, the wines from Bierzo to the rest of the wines. Finally, the discriminant function 3 allowed a good separation between the wines from Toro and Cigales. The physico-chemical parameters that most contributed to the differentiation of wines were the 230 nm. and 280 nm. absorbances, total polyphenols, tannins, flavanols, stilbenes and polysaccharides with a 10 kDa molecular weight average. In addition, the 97.8% of the total wines analysed were correctly classified by the LDA in the corresponding PDO.

Separation of wines by categories was more difficult and only the discriminant function 2 allowed a separation between young wines and those that had long oak aging time (crianza, reserve/gran reserve). The physico-chemical parameters that most contributed to this differentiation were polymeric anthocyanins and total tannins. Only 80% of the wines were correctly classified by wine category.

*Acknowledgements:* This study was supported by a project 2017/721 from "Rural Development Program (PDR) of Castilla y León 2014-2020" (financed with FEADER funds).

IV.P.43

**EFFECT OF THE ADDITION OF MANNOPROTEINS DURING THE PRISE DE MOUSSE ON THE LOSSES OF DISSOLVED CO<sub>2</sub> AND THE FOAM COLLAR OF ROSÉ SPARKLING WINE GLASSES.**

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**Keywords:** Sparkling wine, Mannoproteins, CO<sub>2</sub>, Foam collar

Champagne or sparkling wines elaborated through the same traditional method, which consists in two major yeast-fermented steps, typically hold about 10 to 12 g/L of dissolved CO<sub>2</sub> after the second fermentation in a sealed bottle. Hundreds of molecules and macromolecules originating from grape and yeast cohabit with dissolved CO<sub>2</sub>; they are essential compounds contributing to many organoleptic characteristics (such as effervescence, foam, aroma, taste, colour...). Indeed, the second alcoholic fermentation (called prise de mousse) and the aging on lees (which may last from 12 months up to several years) both induce various quantitative and qualitative changes in the wine through the action of yeast, as listed hereafter: development of complex aromas, release of nitrogen compounds and macromolecules (polysaccharides, lipids, nucleic acids) in wine during yeast autolysis. Moreover, the concentration of dissolved CO<sub>2</sub> reached at the end of the "prise de mousse" is also a parameter of importance since it directly impacts the following properties: the visually appealing frequency of bubble formation in the glass, the growth rate of rising bubbles, the tingling sensation in mouth and the aromatic perception of Champagne and sparkling wines. In recent years, much interest has been devoted to better understand and depict each and every parameter involved in the release of gaseous CO<sub>2</sub> from glasses poured with champagne or sparkling wines.

Here, the impact of yeast mannoproteins on the progressive losses of dissolved CO<sub>2</sub> was closely examined, under standard tasting conditions. A Rosé sparkling wine was elaborated according to the traditional method and divided into different batches after the first alcoholic fermentation.

Each wine was then supplemented with distinct preparations of yeast macromolecules before the second fermentation. Progressive losses of dissolved CO<sub>2</sub> concentrations from these Rosé sparkling wines served in standard laser-etched flutes, were monitored, all along the first 10 min following pouring.

The contribution of each yeast preparation to the collar height and to the bubble size was also evaluated under the same tasting conditions.

## IV.P.44

## EFFECT OF THE WINEMAKING PROCESS ON THE VOLATILE COMPOSITION AND AROMATIC PROFILE OF MATURANA BLANCA WINES

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**Keywords:** Maturana blanca, winemaking techniques, sensory analysis, volatile composition

Maturana blanca *vitis. vinifera* L. var is the most ancient grape variety cited in La Rioja, by the year 1622. However, it is nowadays a minority variety, which has been recently recovered and authorized by the Regulatory Certification Council of la Rioja (D.O. Ca Rioja). Maturana blanca wines are characterized by low pH and high acidity, and are described as fruity wines, particularly with intense apple, banana and citrus fruits characteristics.

Due to its low production, Maturana blanca is used by only a few D.O. Ca certified wineries to mix with other white varieties; however, the interest on the production of varietal wines with Maturana blanca is significantly increasing in order to diversify the wine production of the region.

This study describes the volatile composition and sensory properties of wines made with Maturana blanca. Moreover, it compares the effect of different winemaking techniques: traditional winemaking, elaboration with prefermentative maceration, and, finally, fermentation of Matura blanca musts in oak barrels.

Oenological parameters, volatile composition and sensory properties were analyzed in the different elaborations. Wine volatile compounds were determined by gas chromatography with mass detector (GC-MS). Sensory analysis was conducted by a panel of expert tasters.

#### IV.P.45

### ELECTROCHEMICAL CHARACTERIZATION OF GRAPE EXTRACTS OF SEEDS AND SKINS DURING RIPENING OF *VITIS VINIFERA* GRAPES FOR ANTIOXIDANT PROPERTIES DETERMINATION

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**Keywords:** electrochemistry, grapes, ripening, antioxidant properties

*Vitis Vinifera* skins and seeds are an important source of phenolic compounds with antioxidant properties and human health benefits (José Jara-Palacios *et al.*, 2017). Electrochemical methods for measuring the antioxidant properties of foods and beverages are becoming more widely used due to their sensitivity, rapidity, ease of use and due to their minimal environment effects. (Jorge Hoyos- Arbelaez *et al.*, 2015).

Skins and seeds of 3 *vitis vinifera* red grape varieties (Tannat, Merlot and Syrah) at different stages of ripening were extracted with acetone/water (70/30) and the extracts were analyzed by cyclic voltammetry between -300 and 800 mV (vs Ag) at 100 mV s<sup>-1</sup> using disposable single-walled carbon nanotubes screen-printed electrodes.

Three anodic peaks (oxidation) and one cathodic peak were detected and found to depend on the phenolic composition of the extracts (malvidin 3-glucoside in particular).

Antioxidant capacity of the samples was characterized by the total anodic charge. For all skins varieties, the results showed that antioxidant activity was significant before veraison and then decreased during ripening. The antioxidant activity of seeds increased up until veraison where it reached a maximum and thereafter. For example, Syrah skins had a total charge of 18.42 µC before veraison and then decreased during ripening to a value of 10.53 µC. In the case of seeds, the total charge increased from 23.30 µC to 33.51 µC at veraison and then decreased to 13.13 µC at maturity.

Additionally, anodic charges up to 140 mV (for first oxidation peak) corresponding to the most easily oxidized phenolic compounds were also determined.

Finally the measured total anodic charges were correlated with antioxidant DPPH test values ( $r = 0.74$ ) and with total polyphenol content (Folin Ciocalteu test) ( $r = 0.74$ ) with better results for skins than for seeds.

## IV.P.46

**ENVIRONMENTAL EFFECTS AND LOCALIZATION OF THE CONCENTRATION OF CROWN PROCYANIDINS IN VINE****Alicia JOUIN**

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**Keywords:** Crown procyanidins, Grapes, UPLC-UV-QTOF, Quantification

A lot of plants and vegetables contains phenolics compound with a large range of content and composition. In vine, phenolics compound are present in all the different part of the plant, from the leaf to the fruit. A lot of parameters impact the phenolics composition of the grape like the varieties, the climate, the soil or the terroir. Tannins are one of the most important sub-class of phenolic compound in grape as well as in wine. They are involved in the color stabilization of red wine as well as its astringency and bitterness.

Recently, a new sub-class of tannins, called crown procyanidins, have been detected and identified in the wine. The structure of a crown tetramer has been characterized by NMR, and revealed that it was a macro-cyclic structure composed of four epicatechin monomers link together by regular B-type linkages. These molecules exhibited very specific chromatographic characteristics (higher polarity and partial resistance to phloroglucinolysis depolymerisation) compared to regular B-type procyanidins. These new molecules were identified firstly in the wine but originated specifically from the grape skin.

The aims of this study was to evaluate the environmental impact (varieties, maturity level, soil) on the concentration of crown procyanidins and the localization of crown procyanidins (tetramers and pentamers) in all the vine. Firstly, different varieties grapes have been compared. All the grapes varieties were collected from the same experimental vineyard with the same soil and the same climate. Then, accumulation kinetics of the crown procyanidins during grapes maturity, from veraison to harvest, were studied.

Next, the impact of the soil on the crown procyanidins concentration was also evaluated using samples from different vineyard located in the Bordeaux's region. Finally, different part of vine are collected.

All the berries sample were peeled, freeze-dried and then submitted to a solid/liquid extraction prior to the quantification of the crown procyanidin on the UPLC-UV-QTOF.

Depending on the grape variety and on the ripening stages the content of the crown procyanidins in the grape skin exhibit a large range of concentration. Moreover, the type of soil has also an important impact on the concentration of the crown procyanidins in the grape skin. The localization studies showed that crown procyanidins are not only present in the grape skin but also in the leaf. Interestingly the composition of the crown procyanidins in leaf was different from the grape skin. Indeed in vine leaves there are more crown pentamers than tetramer while it is the opposite in grape skin.

IV.P.47

## EVIDENCE FOR MODERATE LOSSES OF DISSOLVED CO<sub>2</sub> DURING AGING ON LEES OF A CHAMPAGNE PRESTIGE CUVÉE

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**Keywords:** carbon dioxide, champagne, aging, cork stopper

A misconception lingers in the minds of some wine consumers that Champagne wines should not age much after the minimum period of 15 months in contact with dead yeasts, known as maturation on lees. It is certainly a myth, as far as the best cuvées are concerned. Dissolved CO<sub>2</sub> being responsible for bubble formation in sparkling wines, keeping it as efficiently as possible in the sealed bottles during aging is therefore a challenge of importance for old vintages likely to mature on lees for several decades. Measurements of dissolved CO<sub>2</sub> were done on an outstanding vertical collection of successive vintages from a prestige cuvée, showing maturation on lees ranging from several months up to 35 years at a constant temperature of 12 °C. Progressive losses of dissolved CO<sub>2</sub> during aging on lees were evidenced and discussed on the basis of a previously developed exponential-decay type model taking into account the main geometrical parameters of both the cork and bottle. The prestige cuvée (with a narrow bottleneck, and sealed with premium natural cork stoppers) was found to hold much more efficiently dissolved CO<sub>2</sub> during aging than a batch of standard Champagne and sparkling wines bottles showing 3 mm wider bottlenecks. By fitting our data with the exponential-decay model, the diffusion coefficient of gas-phase CO<sub>2</sub> through the cork stoppers used to seal this prestige cuvée was found to range between about  $5 \times 10^{-11}$  and  $9 \times 10^{-11}$  m<sup>2</sup> s<sup>-1</sup>.

Moreover, by extrapolating the data well beyond the measurement range, it was suggested that very long maturation on lees of the order of  $76 \pm 19$  years should be needed to decrease the level of dissolved CO<sub>2</sub> in the prestige cuvée below the critical concentration required for bubbling.

## IV.P.48

EVOLUTION OF ANTIOXIDANT PROPERTIES OF SEEDS AND SKINS EXTRACTS DURING RIPENING OF *VITIS VINIFERA* BERRIES**Nawel BENBOUGUERRA**

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*Vitis Vinifera* berries are an excellent source of phenolic compounds. Berry skins and seeds are rich in phenolic acids, flavanols, flavonols, anthocyanins, proanthocyanidins and stilbenes. These fruits are then an important source of bioactive molecules (Nassiri-Asl and Hosseinzadeh, 2016).

Total polyphenol content and antioxidant capacity in the skins and seeds of 3 *Vitis Vinifera* red grape varieties (Tannat, Merlot and Syrah) at different stages of maturation (pre-veraison, close to veraison and after veraison) were investigated. The berry seeds and skins were extracted with acetone/water (70/30). Total phenolic of samples were measured using the Folin Ciocalteu assay and ranged from 12 to 56 mg/g and 91 to 221 mg/g of dry extracts (expressed in Gallic acid equivalent, GAE) for skins and seeds, respectively. The maximum polyphenolic contents were measured at the first ripening stage (pre-veraison) for the skins and at veraison for the seeds.

The antioxidant capacities of the extracts were evaluated by DPPH, ABTS and FRAP assays. The seeds extracts had higher antioxidant properties than skin extracts for all ripening stages: between 2.3 and 3.8 times more at pre-veraison, 3.8 and 13.1 times more at close to veraison and 4.4 and 8.6 at after veraison.

The correlation values calculated for all samples showed that there is a strong correlation between total antioxidant capacity measured by the different assays and the total polyphenol content (Folin, DPPH  $r = 0.82$ ), (Folin, ABTS  $r = 0.73$ ), (Folin, FRAP  $r = 0.82$ ).

The correlation coefficients for skin extracts antioxidant assays and their total polyphenol content (Folin, DPPH  $r = 0.94$ ), (Folin, ABTS  $r = 0.84$ ), (Folin, FRAP  $r = 0.95$ ) were much higher compared to seeds (Folin, DPPH  $r = 0.71$ ), (Folin, ABTS  $r = 0.62$ ), (Folin, FRAP  $r = 0.69$ ). These results suggest that other non-phenolic compound of seeds might be involved in their antioxidant properties.

#### IV.P.49

## EXTENSIVE CHEMICAL CHARACTERIZATION OF GALLNUT TANNIN EXTRACTS USED IN THE WINE INDUSTRY

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**Keywords:** gallotannins, multi-method analysis, chemical structure, molecular weight distribution

Enological tannins from various botanical sources are commonly used in the wine industry to improve red wine properties. These include gallnut tannins which are gallotannins (hydrolyzable tannins), comprised of a glucose core acylated with several gallic acids. The most widely used methods for characterization of gallotannins are based on protein precipitation and on the quantification of gallic acid released after hydrolysis. However, these methods provide no information on the original tannin structures and molecular weight distribution. Mass spectrometry (MS) enables detection of the species present in the sample but cannot be used for quantitative analysis as the relative intensities do not reflect the proportions of the constituents. Structural information and size distribution can be obtained by NMR spectroscopy and size exclusion chromatography (SEC), respectively. In this study, a combination of approaches has been implemented for extensive chemical characterization of gallnut tannin extracts.

Free and bound (released after methanolysis) gallic acid was determined by UHPLC-DAD-MS whereas free and bound (released after hydrolysis) glucose was determined by GC-MS analysis after reduction and acetylation. These results were compared to those determined from 1D  $^1\text{H}$  qNMR spectrum. Other organic molecules were also identified and quantified by NMR experiments. Size distribution and average molecular weight of polygalloyl glucose were evaluated using both gel permeation chromatography and 2D  $^1\text{H}$  DOSY NMR. MS analysis detected di-, tri- and tetra-gallic acid and mono/polygalloylglucose. Methanolysis yielded methyl esters of mono, di- and tri-gallic acid, as expected, but also of galloylglucose derivatives, suggesting that some glucose are linked to gallic acid through ether rather than ester bonds.  $^{31}\text{P}$  NMR analysis of extracts after phosphitylation of the free hydroxyls provided more insights on the linkage positions of both galloyl and glucose moieties.

Comparison of two batches of commercial gallnut tannin extracts using these methods showed differences in the concentration of gallic acid and gallotannins, tannin molecular weight distribution, and proportions of the different constituents. These differences, although subtle, may have an impact upon their properties when used in the wine industry.

Funding from FEDER and Région Grand-Est, in the frame of "Programme opérationnel FEDER/FSE/IEJ 2014-2020" is gratefully acknowledged.

**IV.P.50****FAST AND ACCURATE AUTOMATED METHOD FOR WINE SO<sub>2</sub> FREE ANALYSIS****Liisa OTAMA**

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**Keywords:** Free sulfur dioxide, Discrete analysis, Gallery analyzer, Automated discrete analyzer

Sulfur dioxide (SO<sub>2</sub>) is used as a process control in wine making. It serves many useful functions, like in musts it acts as enzyme inhibitor preventing juice browning. As a microbiological control agent SO<sub>2</sub> functions not only in musts but also in wines, and it prevents the oxidation of finished wines. Total sulfur dioxide, including free and bound sulfur dioxide, is regulated and needs to be reported. Both total and free SO<sub>2</sub> measurements can be automated.

The purpose of this study was to evaluate the determination of SO<sub>2</sub> free in both red and white wines using Thermo Scientific™ Gallery™ automated discrete analyzer. The method is based on the reaction between sulfur dioxide, p-rosaniline hydrochloride and formaldehyde. The concentration of free SO<sub>2</sub> in the sample is calculated automatically from the calibration curve. Both sample types showed very good correlation between the Gallery discrete analyzer and the flow injection analysis (FIA) method. Correlation coefficient was slightly better for white wines than for red wines, respectively R<sub>2</sub> = 0.9863 and 0.9803.

Precision for the red wine samples (N = 40) was 1.8% within run, and 2.1 % between the runs.

This discrete analysis method enables laboratory to fully automate SO<sub>2</sub> analytics and replace the time consuming traditional Ripper and distillation methods.

#### IV.P.51

### FIRST INVESTIGATIONS ON THE OENOLOGICAL POTENTIAL OF FREISA RED GRAPES: PHYSICO-CHEMICAL PROPERTIES OF GRAPES FROM FIVE DIFFERENT PIEDMONT GROWING LOCATIONS AND OF THE PRODUCED WINES

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**Keywords:** Freisa grapes, autochthonous varieties, oenological potential, anthocyanins

*Vitis vinifera* L. cv. Freisa is an Italian native and historic red grape variety, cultivated primarily in Piedmont region (North-West Italy) and genetically related to the renowned Nebbiolo variety. Freisa is strongly linked to a wide South-East Piedmont area and five Designations of Controlled Origin (DOC), some of them present in the region since 1972, evidence its importance: Freisa d'Asti DOC, Freisa di Chieri DOC, Colli Tortonesi DOC Freisa, Monferrato DOC Freisa, and Langhe DOC Freisa.

The main aim of this study was to investigate the oenological potential of Freisa grapes harvested in each of the five Piedmont above-mentioned designations, and sorted according to density in three classes (1100, 1107, 1115 kg/m<sup>3</sup>) corresponding to three different ripeness degree. Furthermore, the respective wines were produced from unsorted grapes. Grape mechanical properties were assessed by texture analysis, while the content of main phenolic compounds was determined by spectrophotometric and HPLC methods directly on wine or after grape skins and seeds extraction in wine-like solutions.

Results showed that Freisa grapes are characterized by a satisfactory skin hardness (> 0.8 N), a high content of berry skin and seeds flavanols (> 2500 and > 1200 mg/kg grapes, respectively), and relatively high anthocyanins richness (> 800 mg/kg grapes), even if it is prevalent in di-substituted forms (> 56% of total forms). Despite of that, phenolic ripeness parameters showed that the extraction of anthocyanins from the skin into the must is acceptable (EA% > 35%) while, on the contrary, the release of tannins from the seeds is relevant (Mp% > 70%). For this latter reason, Freisa wines were produced with the removal of seeds after 72 hours from the beginning of the fermentation process in order to have balanced wines for bitterness and astringency. Furthermore, the effects induced by growing zone or ripeness degree on Freisa grapes and on produced wines were studied. About berry skin texture parameters, data showed that the hardness parameters ( $F_{sk}$  and  $W_{sk}$ ) were unaffected, while skin stiffness ( $E_{sk}$ ) and skin thickness ( $Sp_{sk}$ ) were correlated to both environmental and ripeness parameters, as well as the total skin anthocyanins and flavonoids content. The content of oligomeric and polymeric flavanols is prevalently variety dependent. Differences between zones were found also in the produced wines, particularly for color traits.

## IV.P.52

## FLAVONOID AND COLOR MARKERS FOR DIFFERENTIATING BETWEEN MONOVA-RIETAL RUFETE WINES AND MIXTURES WITH TEMPRANILLO GRAPES IN D.O.P. SIERRA DE SALAMANCA

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**Keywords:** wine authenticity, grape variety, colour, flavonoids

Consumers and vine growers are more and more interested on autochthonous grape varieties. The former ones are in search of wines with features different from "standard" wines, which are consequence of the global use of a few grape varieties. The latter ones are in search of varieties that would be better adapted to the incoming global climate change scenario. Furthermore, local authorities are also betting on their re-emergence as a way to improve the development of rural regions. In La Sierra de Francia region (Salamanca, Spain), an autochthonous *Vitis vinifera* L. red grape variety, Rufete, is one the flagship varieties that led to the recognition of the D.O.P. Sierra de Salamanca in 2010. Previous studies on the flavonoid composition of Rufete grapes [1,2] have shown the potential of this variety and the wineries belonging to this D.O.P. are interested on taking advantage of its tipicity and make quality wines. Nowadays most of them are producing and marketing 100% Rufete wines, but also wines made from mixtures between Rufete and Tempranillo grapes in order to obtain wines with complementary features. The objective of the present work was to study the CIELAB colour parameters, flavanol, flavonol and anthocyanin compositions of commercial wines from D.O.P. Sierra de Salamanca made from 100% Rufete grapes and compare them to those determined in wines made in the same wineries from different Rufete and Tempranillo mixtures. Wines from different vintages (from 2014 to present) have been analysed by HPLC-DAD-MSn (anthocyanins and flavonols) and by HPLC-MS<sup>2</sup>-MRM (flavanols) [1,2]. These analyses have allowed the comparison between the phenolic potential of Rufete grapes determined in previous works [1,2] and the phenolic composition of these commercial wines. This comparison has made possible, in turn, the establishment of flavonoid markers of Rufete grapes that are still present in monovarietal Rufete wines. Furthermore, the study of wines made from different mixtures between Rufete and Tempranillo grapes has allowed the determination of the percentages of Tempranillo from which the tipicity of Rufete (from the point of view of the flavonoid composition) is affected.

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*Acknowledgements:* Thanks are due to the D.O.P. Sierra de Salamanca and its technical director, Miquel Udina for supplying the wine samples.

#### IV.P.53

## HOW DOES GAS-PHASE CO<sub>2</sub> PROGRESSIVELY INVADE THE HEADSPACE OF CHAMPAGNE GLASSES DURING SERVICE?

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**Keywords:** Champagne, CO<sub>2</sub>, Diode laser spectrometry, Tasting conditions

After uncorking a bottle of champagne, dissolved CO<sub>2</sub> can escape from the liquid phase into the form of bubbles. It is the so-called effervescence process. Moreover, CO<sub>2</sub> molecules also inevitably escape by invisible diffusion through the free air/champagne interface which is the main pathway as concerns the loss of dissolved CO<sub>2</sub> during champagne tasting. Moreover, it is well known that the concentration of dissolved CO<sub>2</sub> is a parameter of importance since it directly impacts some sensory properties such as the mouth feel (with both the mechanical action of collapsing bubbles and the chemosensory excitation of nociceptors in the oral cavity). To date and to the best of our knowledge, the pouring step has been poorly investigated. Actually, the action of pouring champagne is far from being inconsequential with regard to the dissolved CO<sub>2</sub> concentration found in the liquid phase. Losses up to about one third of the initial level of dissolved CO<sub>2</sub> were unveiled during the several seconds of the pouring step. Therefore, because the action of pouring decreases the level of dissolved CO<sub>2</sub> found within champagne, a boost of gas-phase CO<sub>2</sub> inexorably invades the headspace above glasses, thus progressively modifying the chemical space perceived by the consumer.

Based on diode laser spectroscopy, a brand new instrument was built dedicated to monitor accurately, both in space and time, the concentration of gas-phase CO<sub>2</sub> in the headspace of champagne glasses. This instrument, namely the CO<sub>2</sub>-diode laser sensor (CO<sub>2</sub>-DLS), allows to measure gas-phase CO<sub>2</sub> over a wide range of concentrations (up to 100%) with a high temporal accuracy. Here, dissolved and gas-phase CO<sub>2</sub> were monitored during the pouring step of champagne bottles. We examined the impact of different tasting parameters (such as the temperature, the volume of champagne dispensed, and the glass shape) on the losses of dissolved CO<sub>2</sub> and on the progressive increase of gas-phase CO<sub>2</sub> in the headspace of glasses during the several seconds of the pouring step. Our experimental results were discussed on the basis of a multivariable model that accounts for the major parameters that influence the loss of dissolved CO<sub>2</sub> and the evolution of gas-phase CO<sub>2</sub> concentration as well, during champagne serving. Finally, we will be able to propose which tasting condition is the most appropriate to prolong the drink's chill and to help champagne retains its CO<sub>2</sub> content (and therefore its effervescence).

## IV.P.54

## EVIDENCE AND ORGANOLEPTIC IMPACT OF LILAC LACTONE IN BOTRYTISED NOBLE ROT SWEET WINES FROM BORDEAUX

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**Keywords:** aroma, wines, noble rot, *Botrytis cinerea*

Completing the work of Schreier et al. (1976), Miklosy et al. (2000), Sarrazin et al. (2008), research carried out in recent years has clearly established the role of lactones in the aromatic expression of candied fruit in noble rot sweet wines (Stamatopoulos et al. 2014, 2016). Thus, this study was carried out with the aim to progress in the characterization of other odorous lactones in these specific wines. Organic extracts of Bordeaux botrytized noble rot sweet wines, expressing candied fruit overripe nuances, were initially fractionated by HPLC. Then selecting HPLC fractions recalling original fruity nuances, which were different from those evidenced with dry white wines extracts, it was possible to select some fractions which were reextracted and analyzed by gas chromatography coupled to olfactometry. While doing so, an odorous zone reminiscent of overripe fruit and floral nuances which coincided with the obtention of a chromatographic peak by mass spectrometry identified as Lilac lactone (2(3H)-Furanone, 5-ethenyldihydro-5-methyl-) a lactone derivative of linalool and previously mentioned in grapes by Schreier et al. 1976. The identification of this lactone was confirmed by coincidence of retention time and mass spectra with that of the pure product. Then, the focus was on studying its sensory impact and quantitative aspects in wine. This lactone was determined by adapting the method proposed by Ferreira et al. (2004). The analyses were carried out on grape juice and wine.

In noble rot sweet wines from Bordeaux, the contents of lilac lactone can reach a few hundred µg/L, i.e. concentration levels above its olfactory detection threshold, which has been established at 53 µg/L in a model solution of composition close to wine, whereas this compound has only been found in trace amounts in the dry white wines of Sémillon and Sauvignon. From these results, it appears that Lilac lactone can contribute to the aroma of sweet wines of noble rot. In addition, the quantitative measurements also allowed us to confirm the major role of botrytisation on the evolution of the concentrations of this compound in grapes and wine. This study also showed differences in concentration levels according to different vintages as well as between plots studied with the same grape variety, Sémillon, on 2 types of viticultural soils.

Schreier et al. 1976 J. Agric. Food Chem., 24, 2, 331.

#### IV.P.55

### IMPACT OF ENVIRONMENTAL CONDITIONS IN VSCS PRODUCTION DURING WINE FERMENTATION BY *SACCHAROMYCES* AND NON *SACCHAROMYCES*.

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**Keywords:** Volatile Sulfur Compounds, Environmental conditions, Modulation, *Saccharomyces*

Currently, the management and the control of the aroma profile of product to meet the consumer's expectations is a major challenge for winemakers. Indeed, offering quality wines with character and style, allows them to stand out and remain competitive in an increasingly globalized worldwide market.

The wine aroma is a complex mixture of thousands of molecules. These aroma compounds can be classified according to technological winemaking steps in three origins: the varietal aromas are present in grape, the fermentative aromas are produced by yeast and bacteria metabolisms and finally, the post-fermentative aromas, originate from chemical reactions during ageing in tanks, barrels or bottles. With regards to fermentative aromas, a wide range of volatile molecules are produced by yeast during fermentation, which may have a positive or negative incidence on the sensory quality of wines. Of these different compounds, the volatile sulphur compounds (VSCs) exhibit a very low detection threshold and often give off-flavours to wines, negatively contributing to their aroma. These molecules originate from the yeast metabolism of inorganic or organic sulphur sources, but a limited information about their synthesis during wine fermentation is available. Thus, the yeast metabolic network underlying the synthesis of these molecules remains poorly elucidated and the effect of different environmental parameters of interest in winemaking (sugars concentration, pH, nitrogen source composition, pantothenic acid or SO<sub>2</sub> availability) is not well understood.

The principal aim of this work is to provide a comprehensive overview of the formation of VSCs during wine fermentation. For this purpose, we selected 40 strains encountered in this environment (including *Saccharomyces cerevisiae* strains and non-*Saccharomyces* species) to evaluate their abilities to produce VSCs (15 analyzed compounds). We showed a large variability between strains in their ability to produce VSCs. Then we investigated the impact of 5 parameters (pH, nitrogen, pantothenic acid, sugars and SO<sub>2</sub> concentrations) on fermentation performances and VSCs production profiles, using a Box-Behnken experimental design. This allowed us to analyze how these parameters modulate the production of VSCs and SO<sub>2</sub>.

Overall, this study, providing new insights on the modulation by environmental factors of the production of VSCs by yeasts, will allow to define efficient strategies to reduce the formation of VSCs during winemaking.

## IV.P.56

## IMPACT OF FOLIAR GRAPEVINE APPLICATION OF INACTIVE DRY YEAST ON AROMA PROFILE OF CHAMBOURCIN WINE

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**Keywords:** Inactive Dry Yeast, Foliar Spray, French-American Hybrid, Wine Aroma

Inactive dry yeasts are *Saccharomyces cerevisiae* byproducts, recovered during winemaking, that are often used to enhance wine aroma and mouthfeel. LalVigne® (Lallemand, Inc.) is an inactive dry yeast that is rehydrated and applied foliarly to grapevines at the onset of ripening (veraison) to quicken ripening, promote even ripeness, increase phenolic maturity, and improve wine aroma and mouthfeel. LalVigne® application has been evaluated on *Vitis vinifera* grapevines in Hungary, Italy, and South Africa.

Sprayed grapevines were observed to have grapes with thicker skins and greater anthocyanin content. Wines produced from sprayed Sauvignon Blanc grapevines had greater perceived fruitiness, whereas control wines were perceived as more green/unripe. Chambourcin is an interspecific French-American hybrid red wine grape commercially grown in much of the eastern United States.

The objective of this research was to evaluate the effect of LalVigne® foliar application on the aroma profile of Chambourcin wines. Chambourcin vines (8-10 years old) were grown on a single bilateral cordon in a commercial vineyard in Arkansas, USA (USDA cold hardiness zone 6b). Four rows of Chambourcin were sprayed with LalVigne® MATURE at veraison and 10 days later (sprayed), and four rows were unsprayed (control), with six rows between as a buffer. About 50 kg of grapes were hand harvested 27 August, 2018 in quadruplicate from both treatments for wine production. The grapes from both treatments had commercially acceptable soluble solids (21.1%), pH (3.6), and titratable acidity (0.58%) at harvest.

The four batches of grapes from each spray treatment were crushed separately, and the grapes were fermented on the skins for five days at 15°C, two batches with and two batches without the addition of tannin (FT Rouge, 800 mg/L) and medium-toast American oak chips (EVOak, 8 kg/t) (additions and no additions treatments, respectively), and pressed. The wines were racked several times, and the fermentation at 15°C completed with a final ethanol content of 10.5% (v/v) for all wines.

The aroma profile was analyzed in March 2019, using a combination of solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS), SPME-GC-olfactometry (GC-O), SPME-GC x GC-MS, and SPME-GC-triple quadrupole-MS (SPME-GC-TQMS). This provided a comprehensive characterization of Chambourcin wine aroma profiles and demonstrated the effect of LalVigne® foliar application on French-American hybrid wine aroma.

IV.P.57

## IMPACT OF MALOLACTIC FERMENTATION ON VOLATILE COMPOSITION OF CHASSELAS WINES

ÁgnesDIENES-NAGY

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**Keywords:** malolactic fermentation, volatile compounds, terroir, wine aging

Malolactic fermentation (MLF) is usually carried out during the vinification of Swiss Chasselas wines to decrease acidity and vegetal note. With the climate change a decrease is observed in the acidity of grape juice and the necessity of MLF could be discussed. However, previous studies show the importance of MLF on the wine fruity and vegetal aromas. The aim of this study is to determine the impact of MLF on the volatile composition of Chasselas wines. Two other parameters were investigated in correlation with MLF, the year and the terroir.

Chasselas grapes from two experimental vineyards (high and low N level) were processed according to a small scale standardized winemaking procedure with or without performing MLF in 2014, 2015 and 2016. Half of the trials received 4 mg/l oxygen one week before bottling, which was carried out similarly for both type of wine. Wines were stored under controlled conditions (temperature and humidity) and analyzed in 2017 for volatile compounds by GC-FID and GC-MS. Classical parameters (alcohol, acidity, pH, SO<sub>2</sub>, ...) were determined by FTIR and colorimetric methods. As the analysis were done in 2017, "year" effect combines both vintage and wine aging effect.

Sensorial analysis of wines after bottling confirmed, that MLF effected not only acidity, but also the vegetal (herbaceous) and lactic aromas, the body and the balance. Wines with MLF were more appreciated by panelists than the ones without it. Fruity, floral and vegetal aromas were more influenced by the year than by MLF. GC-FID and GC-MS analysis show significant changes in the volatile profile of wines due to MLF. Wines which had undergone the MLF contain higher level of lactate esters, ethyl esters of branched organic acids and 2,3-butanediol. Important decrease was observed in the concentration of diethyl malate, hexane 1-methoxy, 2-propanol-1-butoxy, propyl, butanoic acid esters and 2-ethyl furan. However statistical analysis showed, that year had a greater impact on the volatile composition than the MLF.

In fact, the concentration of volatile compounds can change during the storage. We observed an increase in lactate ethyl concentration in the wines 2014 between 2016 and 2017. This results also show that MLF can influence the aging of wines

## IV.P.58

## IMPACT OF OENOLOGICAL TANNINS ON CHEMICAL COMPOSITION AND SENSORY QUALITY OF WINES FROM MERLOT BOTRYTIZED MUSTS DURING WINEMAKING

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**Keywords:** red wines, botrytized musts, oenological tannins, phenolic compounds

Development of grey mold on grapevines (*Vitis vinifera* L.) due to *Botrytis cinerea* contamination is a very common disease under temperate climate that leads to chemical modifications of grape berries.

During winemaking, especially grape crushing, enzymes, such as laccases, are released by *Botrytis* in grape juice. These laccases oxidize phenolic compounds leading to quinones, which provokes negative effects on musts and subsequently on wine quality. One of the most serious damages is color degradation up to oxidasic casse, making wine quality unacceptable. Beside manual selection of healthy grape clusters, the addition of sulfur dioxide (SO<sub>2</sub>) is the main solution to avoid musts degradations by *Botrytis* enzymes. Thanks to their antioxidant and antioxidasic properties, oenological tannins could be a possible alternative to SO<sub>2</sub>.

Thus, the present study proposes to evaluate the capacity of commercial tannins added to botrytized musts to avoid oxidations during winemaking.

Duplicated micro-winemakings were conducted with or without commercial tannins from healthy and botrytized musts obtained from 0%, 20% or 50 % contaminated Merlot grapes with *Botrytis cinerea* (strain 213). Five commercial tannins from different botanical origins and structure were added at 100g.hL<sup>-1</sup>: grape seed and grape skin procyanidins, quebracho profisetinidins, oak ellagitannins and nutgall gallotannins. Samples were taken at different stages of the winemaking process and analyzed for their oenological parameters, spectroscopic and CieLab color parameters, total phenolic compounds contents, anthocyanins composition, monomers and dimers of flavan-3-ols concentrations, antioxidant capacity (ORAC and DPPH) and laccase activity. Sensory evaluations (triangle and ranking tests) were also performed on 3-month-wines to highlight differences between tannins protection against oxidation.

All measured variables were analyzed with a Principal Component Analysis (PCA). Results clearly show groups corresponding to the main steps of winemaking and the different percentages of botrytization. Another PCA including only post-malolactic fermentation variables shows slight differences between tannins, according to the botrytisation rate. Chemical results and sensory analyses highlight a differential impact of added tannins on oxidation damages protection.

IV.P.59

## IMPACT OF WINEMAKING TECHNOLOGIES ON THE STILBENE CONCENTRATION IN WINE

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**Keywords:** Nanovinification, Thermomaceration, Stilbenes, Biodynamic

Little is known about the release of stilbenes from grapes during the winemaking process. In this study, we examined in nanovinifications the effect of nine different winemaking processes on stilbene extraction and composition in wine. We studied 5 different types of maceration (thermovinification, hot maceration, cold maceration, enzymatic maceration, and rosé wine), and 3 additional procedures with moderate sulphites addition: organic wine, biodynamic wine, and sulphite-free wine. All these winemaking processes were compared to a traditional vinification. We also studied the impact of the filtration process on the stilbene composition for all the nine wines. The contents of stilbenes were analyzed by UHPLC-QqQ.

The macerations with high temperature (thermovinification and hot maceration) provided the highest extraction of total stilbenes, while, as expected, rosé wine provided the lowest content. Moreover, the profile in stilbenes was dependent on the type of maceration: resveratrol was the main stilbene for all the processes except for the high temperature macerations, where cis-piceid was the main compound. Hot maceration and thermovinification seem to promote the isomerisation to the cis-forms of stilbenes. Concerning the filtration step, there was a reduction for the stilbenes content in every winemaking process. This effect was specially marked for the wines from thermovinification..

Our results show valuable information about the influence of the winemaking procedure on the stilbene content and profile in wines.

## IV.P.60

## IMPROVED METHOD FOR HPLC ANALYSIS OF WHITE WINE PROTEINS

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**Keywords:** Proteins, HPLC, Phenolic compounds, interference elimination

Although wine proteins are generally found in very low concentrations, their study has a great interest for oenological science for several reasons. On one hand some proteins may precipitate due to lack of stability (Waters *et al.*, 1991; Esteruelas *et al.*, 2009). On the other hand, the treatments for avoiding this problem, mainly bentonite fining, can affect seriously the wine quality (Ledoux and Dubourdieu, 1994). In that sense, some authors have found that treatment with very high levels of bentonite can have negative effects on wine aroma (Lubbers *et al.*, 1996) and mouthfeel (Guillou *et al.*, 1998). Furthermore, some authors have found that there is a close relationship between protein concentration and foam quality in sparkling wines (Malvy *et al.*, 1994; Medina-Trujillo *et al.*, 2017).

Our research group has proposed some years ago for extraction and an HPLC analysis method of wine proteins (Canals *et al.*, 1998). We propose now an improvement of this procedure which enables to eliminate most of the phenolic compounds bound to proteins. Since phenolic compounds also absorb in the UV range, their presence can cause an overestimation of its concentration.

The procedure consists in dialyzing the wine three folds in front of increasing ionic strength solutions of ammonium acetate prior to the injection on the HPLC device. The chromatograms are monitored at 230 nm for estimating proteins, at 320 for estimating the presence of hydroxycinnamic acids and at 420 nm for estimating yellow pigments (flavonols). The results clearly show that this procedure allows eliminating all the phenolic compounds bounded to proteins avoiding their overestimation.

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IV.P.61

IMPROVING GRAPE SKINS PROANTHOCYANIDIN ANALYSIS AND ISOLATION

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**Keywords:** proanthocyanidins, analysis, CPC, grape skins

Proanthocyanidins are a large kind of phenolic compounds that can be classified as a function of their polymerization degree or the identity of the monomeric units they are formed of.

In grapes and wines five different units are present, catechin, epicatechin, gallic catechin, epigallocatechin and epicatechin-3-O-gallate. The combination of these different subunits and the different kind of link (A or B linked subunits) makes the potential number of compounds huge and since their characteristics are similar, their analysis is quite complex, especially when the polymerization degree is increased.

In grape skins the five different units are present, while in grape seeds gallic catechin and epigallocatechin are not found. When the optimal maturity level of grapes is achieved, the proportion of prodelphinidins in grapes skins is higher, and also the wines obtained are more qualitative, with better organoleptic characteristics, especially regarding astringency and bitterness. Nevertheless, the characterization of the properties of these compounds is a difficult task due to the lack of commercial standards and the difficulty to isolate them from natural sources.

The aim of this work is to improve the isolation and the analysis of skin proanthocyanidins.

For the isolation, CPC (centrifugal partition chromatography) is being used. This methodology allows the separation of compounds without the loss of compounds since there is not a solid phase that would retain a part of them. Several mixtures have been tested in order to achieve better separation of the compounds of interest.

The fractions obtained after CPC are identified using an HPLC-MS (QTOF). Several solvents and gradients are in use in order to improve the separation of the compounds and therefore allow better identification.

This study is the first step in order to achieve an efficient isolation methodology that would allow obtaining these compounds in enough quantity and with an optimal purity to carry out characterization studies.

## IV.P.62

ISOLATION OF EIGHT NEW TRITERPENOIDS FROM *QUERCUS PETRAEA*, IDENTIFICATION IN WINES AND SPIRITS AND SENSORY ASSESSMENTMarine GAMMACURTA

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**Keywords:** wine taste, sweetness, oak wood, LC-HRMS

The composition of wine as well as its sensory properties evolve over time and particularly during barrel aging. The sensory modifications that occur during barrel aging are mainly due to the supply of volatile and non-volatile compounds from oak wood. Indeed, many studies have isolated and identified wood compounds responsible for the modification of aroma, color stabilization, and somatosensory perceptions. Besides these well-known phenomena, an increase in wine sweetness also occurs during barrel aging. On a molecular level, this has been partially explained by the recent identification in oak wood of sweet triterpenoids called quercotriterpenosides (QTT) I, II, III, and VI.

It is well known that natural biosynthetic pathways generally induce structural diversity among secondary metabolites. For a given molecule, isomers or derivatives can be produced in the same plant and might develop similar or more valuable taste properties. Thus, in this study oak wood extracts were screened by LC-HRMS to search for structural analogues to QTT on the basis of their putative empirical formulae. Targeted purification of the analogues using several complementary separation techniques (liquid-liquid extraction, SPE, CPC, and preparative HPLC) was guided by LC-HRMS. The structures of the isolated compounds were assigned by 1D/2D NMR experiments.

Eight new triterpenoids, one known genin, and two known functionalized triterpenoids were isolated from a *Quercus petraea* heartwood extract. Sensory analyses were performed in a non-oaked wine on the pure compounds. Except for two compounds, all molecules exhibited a sweet taste at 5 mg/L that was particularly intense for 3-O-galloylseric acid and arjungenin. Using LC-HRMS, all compounds were observed in an oak wood extract and in oaked red wine and cognac. They were also semi-quantified in several samples of sessile (*Q. petraea*) and pedunculate (*Q. robur*) oak wood extract. All compounds were found in quantities significantly higher in sessile than in pedunculate oak wood. These results support the hypothesis of their contribution to the increase in sweetness during oak aging. Besides, the quantitative assays suggest that these new triterpenoids can be used as chemical markers to identify the species of oak used for cooperage.

IV.P.63

LIGNANS IN SPIRITS: CHEMICAL DIVERSITY, QUANTIFICATION, AND SENSORY IMPACT OF (±)-LYONIREBINOL

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**Keywords:** Lignan, bitterness, taste-active compound, quantification

During barrel aging, spirits undergo organoleptic changes caused by the release of aroma and taste compounds. Among the oak wood nonvolatile compounds, studies have revealed the bitter properties of lignans, such as (±)-lyoniresinol, and their contribution to wine taste. Yet, the sensory properties of a molecule, and in particular its detection threshold, can be strongly influenced by the nature of the matrix from a gustatory point of view. Consequently, the contribution of lignans to spirit taste cannot be presumed from the results obtained in wine. A better knowledge on oak wood and spirits composition appears particularly interesting to improve the quality of these products with high economic interest. Based on these observations, the present work aimed at studying the occurrence of oak wood lignans in spirits and their taste contribution.

First, samples of commercial spirits were screened by liquid chromatography-high resolution mass spectrometry (LC-HRMS) to search for the presence of targeted oak wood lignans. Considering the strong bitterness induced by (±)-lyoniresinol, the detection threshold of this lignan was established in a spirit matrix at 2.6 mg/L. Finally, after development and validation of an LC-HRMS method, lyoniresinol was quantified in various samples of commercial and experimental spirits in order to investigate its sensory impact along with the influence of oenological parameters on its content. Results showed that a large number of analyzed samples contained lyoniresinol concentrations higher than this detection threshold. These results suggested a significant effect of this lignan on bitterness of spirits. Appreciable variations were also observed in commercial spirits, which could be related to differences in barrel aging processes.

So, this study focused on the use of analytical and sensory techniques to highlight the presence of lignans in spirits and the importance of lyoniresinol. More generally, this work brings new insights into and a better understanding of the molecular origin of spirit taste.

**IV.P.64****LONG TERM EFFECT OF WINE CLOSURE OTR ON AROMA COMPOSITION OF SAUVIGNON BLANC WINES: A 10 YEARS STUDY****Alexandre PONS**

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**Keywords:** wine, flavor, aging, closure

While some wines are stored for a short time, many premium wines undergo a long time of bottle storage for maturation - for several years or decades. During this ageing period in the bottles, the wine changes and matures gradually. The maturation makes wine reach a state in which its sensory quality is expressed at its maximum, after which the quality of the wine starts declining. This evolution is related to the composition of the wine in bottle storage and determined by many factors, one of which is the type of wine closure defined as its oxygen transfer rate (OTR).

The effects of the OTR on the sensory properties and aroma compound distribution of three Sauvignon blanc wines were evaluated during 10 years of bottle storage. Natural cork closure, three microagglomerate corks, three synthetic stoppers and two screwcaps were selected.

To investigate this long term effect, the physicochemical parameters as well as sensorial analysis of the wine samples were collected on a regular basis. Basic oenological parameters as well as chemical marker of the quality and oxidation off flavor (3-sulfanylhexanol and sotolon, respectively) were monitored during this experiment. Additional analysis were performed after 10 years such as furfurylthiol a marker of the bouquet of reduction, and methional associated with oxidation phenomenon during bottle aging.

IV.P.65

MACERATION TIME AND SMOKE MARKERS IN RED WINES

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**Keywords:** Smoke, Guaiacol, 4-methylguaiacol, red wine

Many viticultural areas in California have been exposed to wildfire smoke in recent vintages.

As smoke volatiles, when absorbed by grapes, remain mostly localized in skins, it is often advised to shorten macerations in order to mitigate the impact of smoke in red wines. Others believe, however, that smoke compounds migrate extremely quickly from crushed red grapes into juice, rendering such actions ineffective.

During the 2016 and 2017 vintages, juice and fermenting red wine samples from various cultivars (Pinot Noir, Merlot, Cabernet Sauvignon and Petit Verdot) were taken at intervals from when tanks were filled through draining and pressing. Fermentations were initiated and/or completed in glass bottles, without any further skin contact. The end results were series of wine samples from light rosé to dark red, mimicking the wines that would have been obtained if the tanks had been drained at the time of sample collection. These samples were analyzed for polyphenols by UHPLC, and for the main markers of smoke impact (guaiacol and 4-methylguaiacol) by HS/SPME/GC/MS/MS.

Results obtained indicated that the full extraction of smoke derived compounds was completed within two to three days, usually before full extraction of anthocyanins. This is in agreement with anecdotal evidence that making rosés from smoke-exposed grapes often fails to prevent the occurrence of smoke characters in these wines.

As the extraction of tannins is normally delayed compared to the extraction of anthocyanins, results also showed that shortening red wine macerations, while bearing the cost of producing very different wines from a composition and stylistic perspective, appear unsuccessful at alleviating smoke characters.

## IV.P.66

**MATRIX EFFECT OF FERMENTED PRODUCTS AND STABILITY OF MELATONIN AND OTHER L-TRYPTOPHAN DERIVED COMPOUNDS AT DIFFERENT STORAGE CONDITIONS**Ana M TRONCOSO

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**Keywords:** sample treatment, storage, indolic compounds, bioactive compounds

Melatonin (N-acetyl-5-methoxytryptamine) is a bioactive compound that is synthesized by yeast during alcoholic fermentation (Rodríguez-Naranjo *et al.*, 2011) using the amino acid tryptophan as a precursor. Its occurrence has been described in fermented products such as wine, beer, synthetic medium and so others at trace levels (Fernandez-Cruz *et al.*, 2017; Maldonado *et al.*, 2013; Rodriguez-Naranjo *et al.*, 2011). Due to the complexity of food matrices and the low amounts of MEL and L-TRP-derived compounds reported on literature it is necessary to study the matrix effect on the stability of these compounds during storage conditions. Additionally, sample pre-treatment is required to optimize the analysis of these compounds including a prior filtration step (Boyaci *et al.*, 2015). On the other hand, literature reports different filters used to analyze tryptophan derived compounds such as nylon or PTFE (Iriti *et al.*, 2006; Gomez, Hernández, Martínez, Silva, & Cerutti, 2013).

The aims of this work are (i) to optimize the sample treatment in order to improve the analysis of bioactive indolic compounds in fermented beverages and (ii) to check the effect of different conditions of temperature (4°, -20°, -80 °C) at different of indolic compounds concentration (2 and 200 ppb) during storage time.

Nylon filters retain target compounds such as L-TRP, NA5-HT, TOL and MLT in a great extent (29, 46, 47 and 63% of retention respectively). On the other hand, PTFE filters retain minimum amounts of indolic compounds, being the best option for sample filtration. Concerning stability, samples (beer, fermented must and wine) should not be stored for more than 7 days prior to their analysis.

## IV.P.67

## METHYL SALICYLATE GLYCOSIDES IN SOME ITALIAN VARIETAL WINES

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Glycosides are ubiquitous plant secondary metabolites consisting of a non-sugar component, called an aglycone, attached to one or more sugars. One of the most interesting aglycones, found in grapes and in wine, is methyl salicylate (MeSA). MeSA is an organic ester naturally produced by many species of plants, particularly wintergreens. It is synthesised from salicylic acid, a phytohormone that contributes to plant pathogen defence and some of MeSA glycosides are expected to substitute aspirin due to their long-term effects and fewer side effects. To date nine different methyl salicylate glycosides from plants have been reported (Mao *et al.*, 2014). These methyl salicylate glycosides are mainly spread over the genera *Gaultheria*, *Camellia*, *Polypogon*, *Filipendula* and *Passiflora*. From the sensorial point of view, methyl salicylate has a balsamic-sweet odour that is known as "Wintergreen oil".

Methyl salicylate was found in *V. riparia* grapes by (Schreier & Paroschy, 1980) and in *V. vinifera* sp. (Cabaroglu *et al.*, 1997; Versini, Moser, & Carlin, 2005) and in the Frontenac interspecific hybrid (Mansfield, Schirle-Keller, & Reineccius, 2011). We found that the methyl salicylate glycosides content in Verdicchio wines and in some genetically related varieties (Trebiano di Soave and Trebbiano di Lugana) was very high. In order to understand which glycosides were present in wine, the methanolic extract of Verdicchio wine after SPE was injected into a UPLC-Q-TOF-HDMS, extract of different plants particularly rich in such glycosides, were also injected. We confirmed, using pure standards, the existence in wine of two glycosides: a MeSA 2-O- $\beta$ -D-glucoside and a MeSA 2-O- $\beta$ -D-xylopyranosyl(1-6)- $\beta$ -D-glucopyranoside (gaultherin), we also tentatively identified, for the first time in wine, other diglycosides: MeSA 2-O- $\alpha$ -L-arabinopyranosyl(1-6)- $\beta$ -D-glucopyranoside (violutoside) and MeSA 2-O- $\beta$ -D-apiofuranosyl(1-6)- $\beta$ -D-glucopyranoside (canthoside A), a MeSA 2-O- $\beta$ -D-glucopyranosyl(1-6)-O- $\beta$ -D-glucopyranoside (gentiobioside) and a MeSA-2-O- $\alpha$ -L-rhamnopyranosyl(1-6)- $\beta$ -D-glucopyranoside (rutinoside). Some of this glycosides have been isolated from *Gaultheria* leaves and NMR analysis are underway to provide detail information about the structure. In order to understand if these glycosides are a "peculiarity" of Verdicchio wines and its homologous, 40 different white wines were analysed. The range of concentration for the sum of this 6 MeSA glycosides was very variable from 0 to 300  $\mu$ g/L. As the olfactory threshold of this compound is between 50 and 100  $\mu$ g/L, it is suggested that methyl salicylate contributes to the balsamic scent in old Verdicchio wines.

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## IV.P.68

## MICROOXYGENATION: BEHAVIOR OF DIFFERENT WINE PHENOLIC FRACTIONS

**Purificación FERNANDEZ-ZURBANO**

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**Keywords:** wine, microoxygenation, polyphenols

Micro-oxygenation (MOX) is a widespread technique used in wineries with the purpose of bringing about a positive impact on wine quality, in terms of colour, aroma and texture.

It is known that wines show marked differences in the ability to consume oxygen, which mainly depend on their polyphenolic composition. Among these compounds, Aldehyde Reactive Polyphenols (ARPs) seem to play a relevant role, being ARPs content determinant both in the oxygen initial consumption rate and in the acetaldehyde accumulation [1, 2].

The aim of this work was to investigate the impact of MOX in different wine fractions, both chemically and sensorially, obtained by means of gel permeation chromatography and solid phase extraction in comparison with the same fractions kept in anoxia

As previously, it has been proposed [3] two oxygen consumption rates can be calculated from oxygen consumption data: Initial Rate and Average Rate. Fractions without tannins and acids presented lower oxygen consumption initial rate. Fractions containing both anthocyanins and tannins have a significantly higher average oxygen consumption rate than the fractions of anthocyanins and tannins separately. Micro-oxygenated samples showed lower levels of monomeric pigments and small polymeric pigments, but higher of large polymeric pigments.

The sensory characterization showed that samples in contact with oxygen were described less dry and bitter and more unctuous than samples in anoxia.

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*Acknowledgements:* Funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (projects AGL2014-59840, AGL2017-87373, RTC-2015-3379 and RTC-2016-4935-2). MPSN and SFT acknowledge the MINECO for their postdoctoral (Juan de la Cierva Incorporación 2015: IJDC-2015-23444) and predoctoral fellowship (FPI-2018, Universidad de La Rioja), respectively. LAAE acknowledges the continuous support of Diputación General de Aragón (T53) and European Social Fund.

IV.P.69

## MICROWAVE-ASSISTED EXTRACTION OPTIMIZATION OF PHENOLIC COMPOUNDS FROM AHMAR BOU AMAR RED VINE ALGERIAN LEAVES AND THEIR PHENOLIC PROFILE CHARACTERIZATION BY LC-MS

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**Keywords:** *Vitis vinifera*, phenolic compounds, by-products, microwave assisted extraction

For hundreds of years, grape leaves have been used as a popular food material in Middle Eastern countries, either as fresh or marinated. Thanks to their richness in phytochemicals such as phenolic compounds, leaves may be viewed as a safe alternative functional drink. The exploitation of plant by-products such as grapevine leaves by the food industry is gaining ground and presents indisputable advantages, as it is not only a low-cost source of functional ingredients but also constitutes an environmentally friendly processing route, since it greatly simplifies waste disposal management strategies. Microwave assisted extraction (MAE) was investigated for the extraction of total phenolic compounds (TPC, expressed as gallic acid equivalents GAE) from red vine Algerian leaves. MAE is attractive because it allows for rapid heating of aqueous samples and presents advantages over conventional extraction techniques, such as improved efficiency, reduced extraction time, lower solvent consumption, and higher selectivity toward target.

The optimization of the methodology was conducted by using the response surface methodology (RSM) developed by Box-Behnken design (BBD), based on a preliminary studies of conditions affecting TPC extraction such as solvent concentration, microwave power, time and ratio. The optimal MAE processing parameters were 34% (v/v) ethanol concentration, 47s irradiation time, 500 W microwave power, and 1:40 (w/v) material to solvent ratio. Under these conditions, the recovery of TPC was  $53.9 \pm 2.3$  mg GAE/g. The antioxidant activity of the extract obtained was determined by different methods: FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS essay. Twenty-four phenolic compounds were identified and quantified by LC-QqQ in the extract. The major components were Quercetin glucuronide, Quercetin galactoside, Kaempferol glucoside, Quercetin3 rutinoside and Kaempferol glucuronide.

The results of the present study suggest that the Algerian red vine leaves could be a promising source of effective antioxidants.

**IV.P.70****MONITORING ELLAGITANNIN EXTRACTION FROM DIFFERENT TYPES OF BARREL WOODS****Marianthi BASALEKOU<sup>1</sup>**Christos Pappas<sup>2</sup>, Petros Tarantilis<sup>2</sup>, Yorgos Kotseridis<sup>1</sup>, Kleopatra Chira<sup>3,4</sup>, Pierre Louis Teissedre<sup>3,4</sup>, Stamatina Kallithraka<sup>1\*</sup>

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**Keywords:** Barrel, Ellagitannins, Wine, Maturation

Ellagitannins are a group of compounds, quite simple in structure but very important in winemaking due to their relatively high oxidation potential and sensory impact on the final product. Today, big emphasis is being given by researchers to C-glucosidic ellagitannins, specifically vescalagin and its C-1 epimer castalagin, but also to the lyxose/xylose-bearing monomers grandinin and roburin E, the dimers roburins A and D and the lyxose/xylose-bearing dimers roburins B and C as they have been found not only to alter the sensory character of a wine but also to take part in chemical reactions with wine phenols. In this project, all six monomeric ellagitannins have been identified and quantified in red and white wines maturing in barrels made from French oak, American oak, Acacia, Chestnut and Inox tanks with French oak sticks. Ellagitannin composition was determined after column fractionation, and ellagitannin quantification was performed after analysis on a High Pressure Chromatography consisting of a UV-Vis detector, coupled to Mass Spectrometry. The results reveal a different pattern of extraction depending on the type of wood, but also differences in the profile of the dominant monomers according to the type of container and the time of the extraction, as well as a total absence of ellagitannins in the case of barrels made of Acacia wood. These findings can be used to help winemakers decide on the type of wood most suitable to mature a red or white wine but also on the optimum time of contact for each, as ellagitannins correlate very strongly with the perceptions of astringency and bitterness.

IV.P.71

NEW DEVELOPMENTS ON VOLATILE EXTRACTION: APPLICATION OF VASE FOR THE DETECTION OF AROMA COMPOUNDS IN OAK WOOD BY GC-O-MS

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Wine maturation in oak barrels is an important step in the maturation of quality wines. In fact, oak wood develops several aromatic nuances through its toasting which can be released into the wine during this step. A great deal of work has been performed in order to identify the wood-derived volatile compounds that contribute to wine aroma (e.g., whisky-lactone, eugenol, guaiacol, vanillin). However, these compounds only partially explain oak wood contribution to wine aroma.

The method of extraction plays a critical role in the characterization and identification of chemical species in complex matrices. Numerous extraction methods have been thoroughly described in the literature for the analysis of odorous volatile compounds.<sup>1</sup> Among them, liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase micro-extraction (SPME) are most commonly utilized.

This presentation will focus on the latest achievements in the field of aroma extraction with a particular emphasis on a new technique known as vacuum assisted sorbent extraction (VASE, Entech Instruments) or Sorbent Pen™.<sup>2</sup> It enables the extraction of volatile to semi-volatile compounds onto an adsorbent polymer under vacuum.<sup>3</sup> The aim of this study was to compare traditional liquid-liquid extraction to VASE through gas chromatography coupled to olfactometry and mass spectrometry (GC-O-MS). These two extraction methods were applied to the analysis of oak wood samples resulting from several toasting intensities. Various wood species were also investigated.

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## IV.P.72

## NEW FERTILIZATION TREATMENT IN VINE USING WINE DISTILLERY COMPOST: INFLUENCE ON AROMA PROFILE OF WINES

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**Keywords:** Chelva grapes and wines, wine distillery compost, volatile compounds, CG-MS

The high generation of organic wastes together with the increasing interest in developing a sustainable agriculture convert the recycling of these materials as source of organic matter and nutrients in a good option of management. A field experiment was established during 2017 to evaluate the use of a compost made from wastes from the winery and distillery industry in Chelva vine traditionally grown in Castilla-La Mancha region, area where these wastes are generated.

A randomized complete-block design was used with four treatments consisting on three doses of compost: 1 (D1), 2 (D2) or 3 (D3) kg compost per linear meter of plantation and a control (D0) without compost application and the aroma profile of wine was studied by chemical and sensory techniques. For making wines, laboratory fermentations were carried out according to traditional winemaking process for white wines. Volatile compounds were isolated using SPE technique and then analyzed by GC-MS. Sensory aroma profile of wines was evaluated using a trained panel of ten assessors. A total of 81 volatile compounds were identified and quantified in studied wines. Significant differences were found between control wines and wines elaborated with grapes fertilized, in general the fertilization treatment increase the concentration of volatile compounds especially when the dose is 2 Kg/m linear related with floral and fruity notes. It can be seen that when the dose is of 3 Kg/m linear increase the concentration of C6 compounds related with green notes of the wine. The application of compost derived from winery and distillery wastes resulted in slight increases the concentration of varietal aroma compounds related with the aromatic typicity of wines principally when the dose of compost corresponding to 2 kg of compost per linear meter.

IV.P.73

NEW STILBENE DERIVATIVES PRODUCED BY OXIDATIVE COUPLING IN WINE

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**Keywords:** wine, stilbene oligomers, hemisynthesis, oxidation

*Vitis vinifera* (grapevine) stilbenes are a group of secondary metabolites found in various parts of the plant and in grapes. Consequently, several stilbenes like piceid and resveratrol are found in wine.

Their concentration in wine can mainly vary according to the cultivar choice and the winemaking process. However, due to their phenolic structure, stilbenes can be altered under high temperature or oxidative conditions, leading to the formation of other stilbene derivatives [Shingai *et al.*, 2011]. Hence, the goal of this study is to investigate the presence of these derivatives in a wine matrix. In attempt to obtain these compounds, we proceeded to a hemisynthesis of stilbene derivatives from (*E*)-resveratrol and (*E*)-piceid in ethanol by oxidative coupling using metals. The compounds formed were identified by MS and NMR spectroscopy. Moreover, we evaluated the anti-inflammatory effects of the stilbene derivatives by studying their ability to prevent lipopolysaccharide (LPS)-induced upregulation of NO and cytokines production in RAW 264.7 macrophage cell line.

Finally, in a preliminary study, these compounds were identified in a wine solution model with a range of different concentrations of piceid and resveratrol. These stilbene derivatives could serve as indicators of wine quality and conservation conditions.

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## IV.P.74

**OLFACTOMETRY PROFILE AND EVALUATION OF ODOR-ACTIVE COMPOUNDS IN CHASSELAS****Pascal FUCHSMANN**Mireille Tena Stern<sup>1</sup>, Pascale Deneulin<sup>2</sup>, Ágnes Dienes-Nagy<sup>1</sup>

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**Keywords:** Volatile compounds, VIDEO-Sniff, Chasselas

Appreciation of a wine is chiefly influenced by the first perception of its aromas. On one hand, sensory analysis aims to characterize wine aromatic profile with specific descriptors and, on the other hand chemical analyses identify the composition of volatile compounds. Gas Chromatography-Olfactometry-MS/PFPD offers the opportunity to combine these two worlds together by connecting odor perception and chemical identification of volatiles compounds in a complex mixture such as wine.

In the present work, eight Chasselas wines from different regions of Switzerland were studied. First, volatile organic compounds were identified by gas chromatography (GC). Volatile compounds were extracted by headspace In-tube Extraction (HS-ITEX), identification was realized using mass spectrometry (MS) and specific detection for sulphur compounds by Pulsed Flame Photometric Detector (PFPD). Simultaneously, samples were analyzed with olfactometry detection using the human nose as odor detector (GC-O). During the same period, the wines were tasted and described by a sensory panel.

Chasselas wine is very popular in the French part of Switzerland. It is describe with attributes like freshness, delicate fruity, floral and mineral flavors. Despite this vine variety is generally described as non-aromatic, more than 200 volatile compounds were detected in these wines. Moreover, panelists perceived 60 odorants during the GC-O using vocabulary-intensity-duration of elementary odors by sniffing methodology (VIDEO-Sniff). Odor attributes mention by the panelists were classified into ten different families (buttery-cheesy, empyreumatic, floral-fruity, green-fatty, malty-chemical, meaty, spicy, nutty, sulphuric and earthy-undergrowth).

The olfactometry combined with GC-MS/PFPD revealed the presence of twenty-six esters, four aldehydes, three sulfur compounds, nine carboxylic acids, two lactones, eleven alcohols and five ketones. Fruity aroma perceived into these wines may be provide by the following volatile compounds: 3-methylbutan-1-ol, phenylethyl alcohol, 2-methyltetrahydrothiophen-3-one, isoamyl acetate, methyl hexanoate, 2-hexen-1-ol, ethyl-3-methyl butanoate, 2-phenylethyl acetate, ethyl acetate, cis-3-hexenol, ethyl octanoate, oct-1-en-3-one and ethyl hexanoate.

IV.P.75

PHENYLALANINE AND/OR METHYL JASMONATE GRAPEVINE FOLIAR APPLICATION TO ENHANCE GRENACHE GRAPE AROMATIC COMPOSITION

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**Keywords:** foliar application, HS-SPME-GC-MS, varietal aroma, *Vitis vinifera*

Grape aromas are compounds that come from the secondary metabolism of the plants and are directly related to grape and wine quality. Several studies have shown that the foliar application to the vine of phenylalanine (Phe), methyl jasmonate (MeJ) and a mixture of both (Phe + MeJ) can increase the synthesis of some secondary metabolites. The effect of these treatments on the grape phenolic compounds and amino acids content has been studied, but not on the grape aromatic composition. Therefore, the aim of this work was to study the effect of the foliar application of Phe, MeJ and Phe + MeJ on the Grenache grape volatile compounds. The grape volatile composition (terpenes, C<sub>13</sub> norisoprenoids, benzenoid compounds, esters, and C6 compounds) was determined by HS-SPME-GC-MS. The results showed that the MeJ and Phe + MeJ treatments increased the total content of terpenes in the grapes, being the MeJ the most effective treatment, with the content of these varietal compounds up to 98% higher than in the control grapes. On the other hand, all treatments increased the C<sub>13</sub> norisoprenoids content in Grenache grapes, again being the MeJ the most effective treatment, increasing the content of these ones up to 67% respect to the control. For the benzenoid compounds, both the Phe and the Phe + MeJ treatments enhanced their content in grapes, being the Phe the most effective treatment, with an increase of up to 188% respect to the control grapes. All the treatments had a negative effect for the esters found in the grapes. The C6 compounds, which contribute to the herbaceous aromas of the wine, considered as negative notes, did not increase their content in the grapes with any treatment compared to the control. In conclusion, the phenylalanine and/or methyl jasmonate grapevines foliar application is an useful tool in order to improve the aromatic composition of Grenache variety.

## IV.P.76

## PREDICTION OF WINE SENSORY ASTRINGENCY FROM CHEMICAL COMPOSITION

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**Keywords:** wine, astringency, machine learning, tannin activity

Wine appreciation is the result of the interaction between consumer and product 's properties, and these are related to both intrinsic (product 's flavour) and extrinsic (appellation, bottling type, brand...) categories. There are a lot of works focused on studying the impact of extrinsic features on wine appreciation, whereas the impact of intrinsic properties on wine appreciation is less known. In complex systems, such as wine, mouthfeel formation is poorly understood, and in particular, the perception of astringency. The present work aims to predict sensory astringency from chemical composition by machine learning algorithms.

The first hypothesis was that grapes from the same variety (moristel in this case), harvested at different maturity levels and processed with a similar winemaking strategy would yield wines with maximal variability in phenolic composition and most probably in astringency, and minimal aroma variability which would reduce the presence of cross-modal interactions. The second hypothesis was that sensory-chemical relationships between phenolics and astringency do not necessarily have to be linear.

To proof both hypotheses, moristel grapes from different vineyard plots and at different stages of ripening were collected. Eleven different wines were elaborated in 75-litre tanks in triplicate, and further sensory described by the rate-all-that-apply method with a trained panel. The polyphenolic composition was characterised in wines by measuring the concentration and activity of tannins by UHPLC-UV/VIS, the mean degree of polymerisation (mDP) and composition of tannins by thiolysis followed by UHPLC-MS. Conventional oenological parameters were analysed by FTIR and UV-Vis methods. Machine learning algorithms boosted by SDG using DataRobot Platform were applied to build predictive models explaining astringency from chemical composition. The best model was obtained with the support vector regressor (radial Kernel) algorithm presenting RMSE value of just 0.190.

The main variables of the astringency model were the % of procyanidins constituting tannins and ethanol content, followed by other 8 variables related to tannin structure and acidity. Machine learning approaches deem to be essential for progressing in the food science field in general and flavour area in particular.

*Acknowledgements: Funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (projects AGL2017-87373, RTC-2015-3379 and RTC-2016-4935-2). MPSN and SFT acknowledge the MINECO for their postdoctoral (Juan de la Cierva Incorporación 2015: IJDC-2015-23444) and predoctoral fellowship (FPI-2018, Universidad de La Rioja), respectively. LAAE acknowledges the continuous support of Diputación General de Aragón (T53) and European Social Fund.*

IV.P.77

RELATIONSHIP BETWEEN SMOKE MARKERS IN GRAPES AND RED WINES:  
OBSERVATIONS FROM RECENT VINTAGES IN CALIFORNIA

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**Keywords:** smoke, guaiacol, 4-methylguaiacol, grapes

Many viticultural areas in California have been exposed to wildfire smoke in recent vintages.

Despite strong evidence that a significant part of smoke volatiles quickly becomes glycosylated after entering grapes, the analysis of free guaiacol and 4-methylguaiacol in grapes remains a tool of choice to assess the risk of smoke characters appearing in wines. It is important to understand how guaiacol levels in grapes relate to levels found later in resulting wines, since part of the glycosylated smoke volatiles hydrolyze during the alcoholic fermentation.

During the 2015 vintage, we obtained red grapes from multiple fire events and grape varieties. Clusters were destemmed in the laboratory and berries samples were analyzed directly, as well as after acid + heat treatment, in an attempt to hydrolyze glycosylated guaiacols. The remainders were crushed, inoculated and fermented in contact with their skins in small containers. Upon completion of fermentation, resulting "red microferments" were analyzed.

Measured guaiacol levels in grape samples increased only slightly after acid + heat treatment. Both direct and after acid + heat measurements were similarly correlated to levels found in microferments.. Microferments levels were usually 3 to 5 times higher than levels measured directly in grapes.

One outlier was from grapes exposed to smoke only 3 days before they were picked. In this case free guaiacols barely increased post-fermentation. The 2017 Napa and Sonoma wildfires, happening at the tail end of harvest, confirmed this last observation. Guaiacol levels in red wines appeared to be at the maximum 3 times higher than levels found in grape samples. A possible explanation is reduced glycosylation in grapes when exposure to smoke is happening only days before harvest.

Following the 2018 Mendocino Complex fires, which started in late July, red grape samples were collected in vineyards, split for immediate analysis and also crushed and fermented in small containers. Results for free guaiacols in grape and corresponding "red microferments" were very tightly correlated, with increases more in line with what was observed in 2015.

These observations indicate that, taking in account the timing of exposure to smoke, direct analysis of free guaiacols in grapes is a valid tool to predict levels eventually found in red wines.

## IV.P.78

## RESVERATROL IN GRAPES AND WINES OF TANNAT, MARSELAN AND SYRAH FROM URUGUAY

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**Keywords:** resveratrol, stilbenes, grape to wine polyphenols, Tannat, Syrah, Marselan

Resveratrol is a stilbene polyphenol found in several plant families including Vitaceae, where they play function as phytoalexins. It is cited to have properties as a bioactive compound, in medicinal therapies, and as marker of vineyard stress. Its concentrations in grapes and wines is very variable depending on grape variety, growing zone, vintage, etc. Thus, in this work we study the resveratrol contents and profile of grapes and red-wines of *Vitis vinifera* Tannat, Marselan and Syrah from the south of Uruguay of vintages 2015 and 2016. Two harvest dates around technological maturity were considered as well, once in each grape-variety. Both years the vinifications were made in duplicate by traditional maceration, from 10 batches of 70 kg of grapes each. Skin samples were taken before each vinification and freeze-dried. These were extracted three times with a mixture 50:48.5:1.5 of CH<sub>3</sub>OH/H<sub>2</sub>O/HCOOH. The stilbene fraction in these samples and the respective wines (20 samples each) were isolated using SPE C18 cartridges, and injected into an HPLC-ESI-QqQ-MS/MS system equipped with a C18 reversed-phase column (150 × 4.6 mm × 2.7 μm). Identification and quantification were achieved considering the mass transitions (*m/z*) of trans and cis-resveratrol, and their glucosides counterparts trans and cis-piceid, using two MS scan types (EMS and MRM). Wines were analyzed 3 months after winemaking, and 12 months later, and those from 2015 also 24 months after the first analytical determinations. Total resveratrol skin concentrations varied between 17 and 109 mg/kg of grape skin, depending on the grape variety, harvest year, and in Syrah, of grape maturity, since significant increases were detected between harvest. Syrah showed in both years to have the greatest potential for resveratrol synthesis, and only its grapes, presented cis-isomer (all samples). Clearly, such potential was not related to that of other polyphenolic compounds, since Syrah had the lowest total polyphenol content. In wines, resveratrol concentrations varied between 0.6 and 14.0 mg/L, Syrah presenting the highest registers, Marselan the least and Tannat in between. Marselan wines, presented much lower resveratrol concentrations than the expected based on the contents of their grapes, highlighting that extractability could be also decisive of its presence in wine. Interestingly, total stilbene concentrations remained very stable during the analytical period, from wine stabilization up to 24-month later.

IV.P.79

## ROLE OF GRAPE ANTHOCYANIN TRAITS ON THE IMPACT OF OENOLOGICAL TANNINS ADDITION IN THE FIRST STAGE OF RED WINE MACERATION

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**Keywords:** oenological tannins, simulated maceration, anthocyanin profile, grapes

Oenological tannins are widely used in winemaking industry to improve color and aroma wine features. For the first aim, their addition before maceration prevents anthocyanins oxidation, enhances stable pigments formation, and may act as copigmentation co-factor. Generally, oenological tannins can be classified in two main groups, namely hydrolysable tannins and condensed tannins (proanthocyanidins), owing to different antioxidant and reactivity properties.

The aim of this study was to evaluate commercial tannins addition in the first step of maceration of red wine grapes selected according to their anthocyanin profile: generally malvidin prevalent but differentiated by equilibrated acylation (range 8-25%, G1, Montepulciano, Merlot, and Cabernet Sauvignon), high ratio of coumaroyl-derivatives (>25%, G2, Syrah), high ratio of malvidin-derivatives (>50%, G3, Aglianico), high peonin (>15%) and low/absent acylation (G4, Sangiovese), and a peonidin-prevalent group (>45%, G5, Nebbiolo). Grape skins were subjected to simulated macerations in wine-like buffer solutions in presence of different tannin preparations each: two hydrolysable, one mix of hydrolysable and grape proanthocyanidins, one from exotic wood, and four preparations from grapes. Total anthocyanins (TA), color intensity (CI), hue (T), polymeric pigments (TPP), and individual anthocyanin forms were monitored within the first 72 hours of maceration.

Oenological tannins addition led to various effects depending on the variety: in particular, CI was improved in Merlot (G1) by the addition of grape-derived tannins, and in Aglianico (G3) by different tannin preparations. Anthocyanin polymerization (TPP) was favored by using oenological tannins, except for Montepulciano. Proanthocyanidin tannins from grape and exotic wood enhanced color stabilization of Merlot and Cabernet Sauvignon (both G1), respectively. For G2, G3, and G4 varieties, higher TPP was reported for hydrolysable tannins, followed by those from exotic wood for G2 and G3 varieties, and grape-derived proanthocyanidins for G4. In Nebbiolo (G5), a higher polymerization was reached using grape-derived tannins. Notably, exotic wood tannins addition led to a higher percentage contribution of delphinidin, petunidin, cyanidin, and peonidin glucosides in both Nebbiolo (G5) and Sangiovese (G4). Therefore, the anthocyanin profile of grape variety may be considered one of the factors useful for the choice of commercial tannins.

## IV.P.80

**STILBENES FROM *VITIS VINIFERA*: PHYTOCHEMICAL ANALYSIS AND STUDY OF THEIR ANTI-INFLAMMATORY EFFECTS IN LPS-STIMULATED MACROPHAGES****Arnaud COURTOIS**<sup>1,2</sup> Iris Aja-Perez, <sup>2</sup> Stéphanie Krisa, <sup>2</sup> Ruth Hornedo-Ortega, <sup>1</sup> José Ignacio Ruiz-Sanz, <sup>2</sup> Tristan Richard, <sup>2</sup> Arnaud Courtois

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**Keywords:** Stilbenes, Inflammation, Oxidative stress, Macrophages

**Background.** Stilbenes are natural compounds present in edible foods and some beverages such as red wine. They derived from resveratrol that represent the most stilbene studied in the past decades. Some of these monomers and oligomers present anti-oxidant and anti-inflammatory properties. The goals of this study were: (i) isolate and characterize stilbenes from grapevine extracts, (ii) evaluate and compare the anti-inflammatory properties of these stilbenes in Lipopolysaccharide (LPS)-stimulated murine macrophages.

**Methods.** Resveratrol and 19 stilbenes were isolated, identified and characterized from *Vitis vinifera* shoots in our laboratory. Their toxicity against murine macrophage cell line RAW 264.7 was assessed by MTT and neutral red assays. Their potential to limit inflammatory or oxidative responses were evaluated by measuring NO, TNF $\alpha$ , and IL1 $\beta$  release using Griess reagent, ELISA kit, and H2DCFDA fluorescence assay, respectively.

**Results.** Twenty stilbenes from grapevine extracts were isolated and characterized by mass and NMR spectroscopy. Results showed that toxicity of these compounds increased with the degree of oligomerisation, being the monomers the less toxic. Most of them were able to suppress NO release from LPS-treated macrophages except the monomers piceid, astringin, oxyresveratrol and isorhapontin, the dimers ampelopsin A, scirpusin and pallidol, and the trimer myabanol C. At non-toxic concentration, the monomer piceatannol, the dimers  $\epsilon$ - and  $\delta$ -viniferin, and the tetramers hopeaphenol and isohopeaphenol dose dependently suppressed the NO release in LPS-treated cells. The stilbenes were also able to inhibit the TNF $\alpha$  and IL1 $\beta$  production in LPS-treated cells at concentrations inhibiting NO production. Finally, piceatannol,  $\delta$ -viniferin and hopeaphenol inhibited ROS production in LPS treated-cells whereas  $\epsilon$ -viniferin and isohopeaphenol did not.

**Conclusion.** The resveratrol monomer piceatannol, the resveratrol dimers  $\epsilon$ - and  $\delta$ -viniferin, and the resveratrol tetramers hopeaphenol and isohopeaphenol presented the most powerful anti-inflammatory response in LPS stimulated macrophages. This effect was not always correlated with the anti-oxidant properties of the molecules. Nevertheless, those molecules, isolated from *Vitis vinifera*, could represent useful agents for the prevention of diseases involving inflammation or oxidative stress in their pathophysiological processes and could explain some beneficial effects of extracts derived from vine or wine.

IV.P.81

## SURVEY ON THE VARIABILITY IN THE COMPOSITION AND PHENOLIC PROFILE OF THE MAIN MONOARIETAL ITALIAN RED WINES

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**Keywords:** Italian red wines, monovarietal wines, phenolic characterization, technological parameters

Italian red wines are well known for their characteristics and diversity, this last factor being induced by the high number of autochthonous varieties used for winemaking. This study is part of an extensive Italian national collaboration aiming at evaluating by means of different physico-chemical and sensory approaches the diversity of Italian wines. In this sense, we assessed the compositional and phenolic variability of more than one hundred monovarietal red wines, vintage 2016, obtained from wine cellars across Italy. Eleven different grape varieties, well representing a high share of the Italian quality red wine production, were included in the study: Aglianico, Cannonau, Corvina, Montepulciano, Nebbiolo, Nerello Mascalese, Primitivo, Raboso Piave, Sagrantino, Sangiovese, and Teroldego. These wines, directly taken from cellars and not subjected to malolactic fermentation or oak aging, were analyzed for their compositional chemical parameters (alcohol content, net dry extract, residual sugars, acids, pH, glycerol) and for spectrophotometric phenolic parameters (total phenolics, total and monomeric anthocyanins, oligomeric and polymeric flavanols). The experimental design allowed an evaluation of the specific traits of wines produced from a single variety and of the differences among these wines.

Results evidenced an average alcohol content of 13.9 % v/v, without a clear relationship between the ethanol content and the geographical location of the Italian zone considered. With respect to the other cultivars tested, Nebbiolo and Teroldego wines (11 different samples each) evidenced a low in-variety within-group variation for ethanol, net dry extract, and glycerol parameters. Regarding anthocyanins, Teroldego showed the highest contents among the varieties considered, and 47 % of them corresponded to monomeric forms, coherently with the average value of all the varieties studied.

Instead, Corvina and Nebbiolo showed the lowest values of anthocyanin content, and their monomeric forms ratio differed among them (48 % vs 38 %, respectively). The determination of oligomeric and polymeric flavanols confirmed the attitude of some Italian wines to present distinctive characteristics, with Sagrantino, Nebbiolo, and Aglianico achieving the highest values of the study for both contributions.

## IV.P.82

**SYRAH CV. CHILEAN WINES: CHARACTERIZATION OF COLD AND WARM CLIMATE SYRAH WINES THROUGHOUT GRAPE RIPENING.****MARIONA GIL I CORTIELLA**

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**Keywords:** syrah wines, phenolic composition, cold climate, warm climate

Chile is one of the main wine producers from South America. Moreover, Chile has a great diversity of viticultural environments, due to its geographical features. The coast vineyards are considered as cold climate vineyards, when compared with the most inland vineyards, because the influence of the Humboldt Current in the Pacific Ocean. Lower temperatures and more cloudy days delay the ripening of coast vineyards, which also impact on the sensory properties of the wines. Most of the scientific literature regarding warm climate wines is focused on syrah wines, since they are very appreciated by the international market. Moreover, syrah is one of the main red cultivars in the coastal Chilean regions (alongside Pinot noir). Hence, syrah was chosen as the model for this study in order to characterize cold and warm climate Chilean wines.

The aim of this work is to characterize the Chilean wines made from syrah cv. (clone 178) coming from coast (cold climate) and inland (warm climate) vineyards respectively. Syrah grapes were harvested at three different maturity levels for each vineyard (unripe, mature, and overripe grapes), and used for red winemaking by triplicate. Afterwards the wines were chemically characterized.

The amount of total phenols, total anthocyanins, and total tannins increases during grape ripening for both climates. In contrast, the wine color intensity increases from the first to the second harvest, but it decreases again from the second to the third harvest (hence, the wines made from overripe grapes shows lower color intensity than the wines made from mature grapes). Wines from both climates show similar amounts of total phenols. In contrast, the cold climate wines shows deeper color, higher amount of total anthocyanins, and a lower amount of total tannins.

The sensory features of wines from both vineyards are very different, pointing that syrah is a very plastic cultivar, and that it could be a great cultivar in order to enhance terroir typicality.

*ACKNOWLEDGEMENTS:* The authors would like to thank CONICYT-Chile for financial support (RED1170088 and FONDECYT 1181142).

IV.P.83

TARGETED METABOLOMICS STUDY FOR VARIETAL DISCRIMINATION OF WINES ACCORDING TO PHENOL COMPOSITION AND TENTATIVE ELUCIDATION OF PARTICULAR VARIETY-TYPICAL SENSORY FEATURES

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**Keywords:** UPLC-QqQ-MS/MS, wine, phenols, variety

Information on the content and composition of phenols in monovarietal wines is of fundamental importance for adequate wine quality management and control, it is useful for predicting wine sensory properties and oxidative stability, and may indicate wine age and production technology. It may also be used for authentication of varietal origin, as well as a strong point in marketing.

To investigate the possibility of wine varietal differentiation based on phenol composition despite the existing heterogeneity, almost 200 samples produced from grapes of various red and white varieties grown in various terroirs in three Croatian regions were subjected to targeted profiling of phenols by ultra-performance liquid chromatography-triple quadrupole mass spectrometry. Descriptive sensory analysis was performed, including evaluation of varietal typicality, to investigate if there is a relation between variety-typical sensory profiles and certain phenols.

Many of identified phenols were attributed as discriminators between wines. **Plavac mali** was characterized by the most distinguished anthocyanin profile, with generally the lowest concentrations among the investigated red wines. On the other hand, the wines from this variety were generally the most abundant in non-anthocyanin phenols. **Cabernet Sauvignon** wine stood out with the highest values of acetylated to p-coumaroylated anthocyanins ratio and the percentage of the sum of the acylated anthocyanins. **Teran** wine was characterised by the highest concentration of anthocyanins in general, while **Merlot** exhibited the least distinguished profile. As for white wines, those from the region of Dalmatia made from **Pošip** and **Maraština** varieties were generally more abundant in phenols than others. **Malvazija istarska** wines contained the lowest concentration of the majority of flavonols and hydroxycinnamates, while **Graševina** was distinguished by the lowest amounts of flavan-3-ols. The highest concentrations of hydroxycinnamates were found in **Muscat blanc** wines. Linear discriminant analysis resulted in successful differentiation, with peonidin 3-(6"-acetyl)-glucoside and taxifolin as the most potent differentiators among red, and cis-piceid among white wines. Particular varietal typicality concepts were partially confirmed by tentatively relating the concentrations of certain phenols and their possible sensory effects.

*This work has been supported by the UIP-2014-09-1194 (Croatian Science Foundation) and ADP 2017 (Trento, Italy) projects.*

**IV.P.84****THE ANALYSIS OF POLAR ANIONIC PESTICIDES BY A NEW INTEGRATED, ROBUST AND SENSITIVE 'SAMPLE-TO RESULT' IC-MS/MS WORKFLOW****Richard FUSSELL**

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**Keywords:** Anionic pesticides, Suppressed IC-MS/MS, Trace level analysis, Glyphosate

This presentation provides information on the development and validation of a new integrated 'sample to results' workflow for the reliable and sensitive quantitation of polar anionic pesticides and contaminants in food. The workflow is based on the use of high capacity ion exchange columns with post column eluent suppression coupled to a high sensitivity triple quadrupole mass spectrometer (IC-MS/MS). This development is important because polar anionic pesticides and contaminants such as glyphosate and metabolites, perchlorate, chlorate and the like, often occur as residues in food; but are not always included in food safety monitoring programs, simply because they are not 'amenable' to more conventional generic multi-analyte methods.

The introduction of the commonly used Quick Polar Pesticides (QuPPE) Method was a major step forward, but it is still analytically challenging to laboratories almost 10 years later. The absence of a liquid partitioning step and/or solid phase clean-up step, results in 'dirty extracts' containing high concentrations of matrix co-extractives, thus the separation and accurate quantification of analytes in QuPPE extracts is difficult. Analysts attempt to mitigate these issues by the use of labelled internal standards and analysis of a single extract a number of times using different low capacity chromatographic columns with various elution conditions to maximize analyte coverage.

The IC-MS/MS workflow approach enables aggregation of separate methods into a single analysis improving productivity, while the high capacity ion exchange columns can withstand higher sample loading enabling the analysis of lower concentrations of polar analytes in grapes and wine juices.

IV.P.85

THE CONTRIBUTION OF SKIN TO WINE FLAVAN-3-OLS COULD BE OVERESTIMATED BY COMPARING THEIR PROPORTIONS IN PRODELPHINIDINS

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**Keywords:** flavan-3-ols, red-wine, grape to wine polyphenols, Tannat, Syrah, Marselan

Seed and skin flavan-3-ols have structural differences with enological implications. Skins flavanols are based on catechin and epicatechin (procyanidins, PC) together with gallocatechine and epigallocatechine (prodelphinidins, PD), the later absent in seeds. From this, it is possible to estimate the skin and seed relative contribution to wine flavan-3-ol. This method relay on the assumption that PD and PC are similarly extracted during maceration, and that their stability are similar once extracted. To test this, we consider the %PD in skin, but also in grape skin after winemaking (pomace), and in wines over time.

The investigation was made from *Vitis vinifera* Tannat, Marselan and Syrah grapes cultivated in the south of Uruguay in years 2015 and 2016. Each year 10 batches of 70 kg of grapes each were vinified by traditional maceration. Skin samples were taken before and after each vinification and freeze dried. These were extracted three times with a mixture 50:48.5:1.5 of CH<sub>3</sub>OH/H<sub>2</sub>O/HCOOH. The flavanol fraction in these samples and the respective wines (20 samples each) were isolated using SPE C18 cartridges, and injected before and after pyrogallol-induced acid-catalyzed depolymerization into an HPLC-ESI-QqQ-MS/MS system equipped with a C18 column (150 × 4.6 mm × 2.7 μm). Identification and quantification were achieved considering the mass transitions (*m/z*) of the expected flavan-3-ols using two MS scan types (EMS and MRM) employing catechin as external standard. Wines were analyzed 3 months after winemaking, and 12 months later, and those from 2015 also 24 months after the first analytical determinations. In the wines, the %PD did not change significantly over time, indicating comparable stability of PD and PC. However, the %PD of skin-pomace was significantly lower than in skin (12 and 30% respectively) indicating that PD were more extracted than PC.

Supporting this, differences were higher in the flavan-3-ols monomers (31% skin, 7% pomace) than in the extension (30 vs 12%) or terminal units (26-12%), these latest derived from polymers (dimers onward) containing PD and PC units. In summary, the presented data suggest that to obtain a more accurate estimation of skin contribution to wine tannins the preferential extraction of prodelphinidins would be considered. Some dimers and monoglucosides of catechin were just identified in seeds, and could be promising as additional indicators of the origin of wine tannins.

## IV.P.86

**THE EFFECT OF SEVERAL POLYSACCHARIDES AND POLYPHENOLS ON THE RELEASE OF VOLATILE COMPOUNDS AND THEIR EVOLUTION IN THE HEADSPACE OF WINE****Ricardo LOPEZ**

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**Keywords:** wine, macromolecules, headspace, volatile compounds

The aroma typicality of a particular wine is owing to its complicated constituents, not only the volatile compounds that could be directly perceived, but also the non-volatile compounds in the matrix, which can exert a powerful effect on the release of odorants. The nature of the interaction between volatile compounds and wine matrix has been evidenced as result of chemical bindings, such as hydrophobic interaction, hydrogen bonds or covalent bonds. Therefore, the capacity of binding differs not only depending on the physicochemical characteristics of volatile compounds, but also the chemical properties of non-volatile components.

In the present study, commercial samples of gum arabic, carboxymethylcelluloses (CMC), oak tannins and yeast mannoproteins were used to prepare a synthetic wine matrix with a standard composition of common wine odorants from representative chemical families. A previously validated method (1) based on dynamic headspace sampling was applied to study the volatile-macromolecule interactions and the headspace evolution under dynamic conditions.

The present work will show that some of the commercial products based on macromolecules frequently used as enological additives in the wine industry could interact with volatile compounds resulting in a global retention effect. For some volatile compounds, the interaction would influence the evolution of headspace composition not only at the initial release, but also over time. Moreover, the chemical characteristics of the volatile compounds strongly impact the binding capacity, such as the hydrophobicity and chemical structure. According to these results, the studied macromolecules showed differences in binding capacity with some volatile compounds due to their different chemical properties.

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IV.P.87

## THE EVALUATION OF PREBIOTIC ACTIVITY RELATED TO OLIGOSACCHARIDIC FRACTIONS EXTRACTED FROM GRAPE SEEDS AFTER AN ENZYMATIC TREATMENT

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**Keywords:** Grape seeds, Oligosaccharides, Enzymatic treatment, Prebiotic activity

Recent studies highlighted that grape seeds are a putative source of functional compounds such as oligosaccharides [1, 2]. Chemical characterization of these compounds showed both the heterogeneity and complexity of their structures. Oligosaccharides identified may be considered, in some combinations, a novel "functional ingredient" with potential prebiotic activity towards well-known probiotic bacteria (*Lactobacillus acidophilus*), allowing to improve the growth during in vitro fermentation [3]. The aim of this study was to evaluate the prebiotic activity of different oligosaccharidic fractions obtained from grape seeds towards another well-known probiotic bacterium such as *Lactobacillus brevis*. Moreover, after the first extraction, the seeds were subjected to an enzymatic treatment to evaluate the presence of other bound oligosaccharides. A highly complex enzyme system, characterized by an association of pectolytic (pectin lyase, polygalacturonase and pectin methyl esterase) and cellulolytic activity (cellobiohydrolase (C1), beta-endo-D-glucanase (Cx) and cellobiose), was used. Proper oligosaccharides purification has been realized by using an appropriate multistep solid-phase extraction. Residual polyphenols (still present in the matrices and characterized by an antimicrobial effect such as oligomeric and polymeric proanthocyanidinic classes) have been removed during the extractive steps, so avoiding the related interference toward the potential prebiotic effect of the extracts. These oligosaccharidic fractions, extracted with and without enzymatic treatment from grape seeds, showed a significant prebiotic activity on *Lactobacillus brevis*.

*Acknowledgements* The financial support of Progetto Ricerca Locale DSF 2016.

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## IV.P.88

## THE IRRIGATION OF CABERNET SAUVIGNON VINES WITH OZONATED WATER AFFECT THE CHEMICAL COMPOSITION OF WINES

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**Keywords:** ozonated water, irrigation, Cabernet Sauvignon, phenolic and volatile compounds

The policy of Sustainability implies developing new strategies and practices to reduce the use of pesticides, as they have a negative impact on the environment and human health. Among the products that can replace the pesticides used in viticulture, one of the most interesting is ozone as an aqueous solution, for its proven efficacy in fungal diseases of the vine, and because it can be considered as ecological since it does not generate residues. The effect of ozone on post-harvested grapes is well documented, but there is a lack of information on the effect of ozonated water on grapes pre-harvest, and therefore, such effect is unknown on the oenological ability of grapes. One of the possible ways to use ozone in the vineyard is through the irrigation system, which is implemented in most of the vineyards of warmer climate areas. Therefore, the objective of this work was to study the effect of ozonated water, applied through the irrigation system, on the wine quality.

Cabernet Sauvignon vines from Castilla-La Mancha (Spain) were used to apply two different treatments of ozonated water: T1 (irrigation) and T2 (combining irrigation with sprayed) where three applications were made in T1 and six in T2. Grapes at the optimum technological ripening were used and the vinification was conducted by the classical process. After the malolactic fermentation, the oenological and chromatic parameters were determined, as well as a detailed analysis of their volatile and phenolic composition by SBSE-GS-MS and HPLC-DAD respectively.

Both treatments produced a decrease in the alcohol degree and in the fructose and glycerol content. However, in many other parameters analyzed a significantly different effect was observed according to the type of treatment. Among them, there was an increase in the Total Polyphenol Index and the absorbance at 520 nm when T2 was used and a decrease when T1. Consequently, an important increase of the anthocyanins was obtained in T2 wines, both non-acylated and acylated forms. The wine volatile content was also influenced by both treatments, since an increase of ethyl esters (with the exception of ethyl acetate, which decreased), acetates and 2-phenylethanol was observed. Therefore, treatments with ozonated water by combining irrigation and spraying, not only would have an effect on the sanitation of the vineyard, but could also to produce wines with lower alcohol content, better colour and a differentiated aromatic profile.

IV.P.89

## THE MULTI-RESIDUE ANALYSIS OF TRACE LEVELS OF PESTICIDES IN WINE USING A LC-MS/MS

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**Keywords:** Pesticides analysis, Multi-residue method, Trace analysis, LC-MS/MS

### Introduction:

The usage of pesticides is permitted to control, fungi, mites, insect and weeds etc. during the non-organic cultivation of wine grapes. It is known that pesticide residues can transfer from the grapes into the wine, so it is important to monitor wines to ensure that concentrations of any pesticide residues do not exceed regulatory or voluntary maximum levels. Comprehensive analysis of all of the different classes of pesticides requires the use of ion chromatography-, gas chromatography- and liquid chromatography- coupled to mass spectrometry. In this poster presentation we describe the sensitive, accurate and robust determination of hundreds of LC-amenable pesticide residues in wine, in a single analysis.

### Method:

Wine samples were extracted with acetonitrile using the QuEChERS (Quick, Easy, Cheap Effective) approach. Final extracts were analysed using a Thermo Scientific TSQ Quantis triple quadrupole mass spectrometer coupled to a Vanquish Flex Ultra High Performance Liquid Chromatograph. Chromatographic separation of the pesticides was achieved using a Thermo Fisher Scientific™ Accucore aQ column (2.1 x 100 mm, 2.6mm) using gradient elution with 0.1% formic acid/5 mM ammonium formate in water and 0.1% formic acid/5 mM ammonium formate in methanol, respectively. All analytes were eluted within 15 minutes and the injection was 1µL. A TSQ Quantis triple quadrupole mass spectrometer coupled to a Vanquish Flex UHPLC was used.

### Preliminary Data:

The multi-residue method enabled the screening (550+) and quantitation of approximately 300 pesticides in one 15-minute run with polarity switching. Pesticides were unequivocally identified using ion ratios of MS/MS transitions ( $\pm 30\%$ ), and repeatability of retention time ( $\pm 0.1\text{min}$ ) in compliance with the method performance criteria set out in the EU SANTE Guidelines (11813\_2017). All pesticides showed excellent Limits of Quantitation between 0.5 to 10ppb, with satisfactory recovery, precision and linearity during method validation. Retail samples of wines were tested for pesticides. Further details on the workflow, the method validation and analysis of samples will be provided.

## IV.P.90

## THE MULTIPLES CHEMICAL FORMS OF DIMETHYL SULFURE. A POSSIBLE CONTRIBUTION TO THE POLYMORPHISM OF FINE AGED WINES DURING TASTING

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**Keywords:** dimethylsulfure, wine bouquet, bonded aromas

The central role of dimethyl sulfide (DMS) in the expression of the aging bouquet of red wines has been demonstrated. It participates to the expression of the undergrowth/truffle note, to the polymorphism of the aroma during the tasting, to the typical bouquet of Bordeaux wines and in the perpetuity of the fruity character<sup>1,2</sup>. DMS origins are in the vine, its precursor (S-methylmethionine) has been identified and the influence of the nitrogen vine status on its levels have been studied<sup>3</sup>.

The quantification of DMS levels in 48 wines (27 red wines, 9 white wines and 12 rosé wines) using two quantification methods, leads to observe a deviation of quantified levels according to the sample preparation. The levels measured using a room temperature static head space sample preparation associated with GC/FPD<sup>4</sup> are different but highly correlated with the ones measured in the same wines using a swirled and warmed HS-SPME sample preparation associated with GC/MS. The correlation is true only for wines with comparable ages (for red wines) and same color. This deviation is different according to the phenolic composition of wines and with the wine ages. This first result opens the hypothesis of bonded forms of DMS.

The second step was to understand the physico-chemical parameters that influence the bonded forms of the DMS in wines (temperature, pH...). The final step is to propose hypothesis on the mechanism of chemical bonds between DMS and other wine molecules. Chimisorption modelisations have been performed.

This work permits to enhance the knowledge on the DMS levels in the head space of a glass of wine<sup>5</sup>. This could be a part of the revelation of the magic of the aging bouquet of wines<sup>6</sup>.

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#### IV.P.91

## VOLATILE COMPOSITION AND OLFACTORY PROFILE OF VERDUNO PELAVERGA WINE

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**Keywords:** Spicy, Pelaverga, rotundone, wine

The Verduno Pelaverga wine is produced in a small area which includes the municipalities of Verduno, La Morra and Roddi d'Alba near Barolo, in Piedmont. The wine is obtained from Pelaverga piccolo added with a maximum of 15% of non-aromatic red grape varieties suitable for cultivation in Piedmont. Previous studies (Gerbi & Zeppa, 1993; Valenti *et al.*, 1993) revealed that this wine is characterized by intense spicy and fruity aromatic notes. The aim of this work, carried out at CREA Research Centre for Viticulture and Enology, was the deep characterization of the peculiar aroma of this wine, both from a sensorial and analytical point of view.

Five different wines obtained during the 2017 vintage were analyzed by gas chromatographic (GC-qMS), olfactometric (GC-O) and sensorial analysis in order to accurately delineate their aromatic profile. The sensory analysis confirmed previous results further showing that Pelaverga wines are characterized by typical spicy notes (pepper and nutmeg) together with flavors of cherries and dehydrated fruit.

The compositional aspects that best discriminate Verduno Pelaverga from other Piedmont or Italian wines are the presence of significant concentrations of some varietal compounds like sesquiterpenes and volatile phenols such as guaiacol. In this regard, the olfactometric analysis linked, for the first time, the typical spicy notes of Verduno Pelaverga wines to these odoriferous compounds present in concentrations above their respective detection threshold. In particular, guaiacol is a vector for spicy and smoked notes and olfactometry results include it among the compounds with the greatest aromatic impact. Moreover the presence of rotundone, with notes of black pepper, characterized the bouquet of Pelaverga wines in an original way, similarly to what has been shown for wines from Syrah cultivar (Wood *et al.*, 2008).

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## IV.P.92

## CYCLIC VOLTAMMETRY ANALYSIS OF SELECTED RED AND WHITE WINES FROM THE STELLENBOSCH WINE REGION, SOUTH AFRICA

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**Keywords:** cyclic voltammetry, phenols, sensor, tyrosinase, biosensor

Wine contains polyphenols that are responsible for its quality. Moreover, phenolic compounds have antioxidant properties and benefits on human health. Cyclic voltammetry (CV) was the first electrochemical method used for polyphenols characterization and determination of polyphenols content in wine products. Electrochemical methods for evaluating antioxidant activity have emerged in the past decade, as an alternative method to conventional chemical assays. Cyclic voltammetry measures electron-donation capability (redox potential) of antioxidants, which respond to a voltammetric scan according to their redox potential. CV provides a qualitative and quantitative assessment of wine phenolics based on their reducing strength, and charge passed to 500 mV (vs Ag/AgCl).

We will present the quantitative and qualitative analysis of selected red wines and white wines, by cyclic voltammetry, for wines from the Stellenbosch wine region (Western Cape, South Africa). CV was used to monitor the electrochemical behaviour of four phenolic compounds representing the main phenolic groups found in wines and winemaking by-products, namely gallic acid (hydroxybenzoic acid), caffeic acid (hydroxycinnamic acid), catechin (flavanol) and quercetin (flavonol). These compounds have in common a catechol moiety believed to be the electrochemically active group.

Furthermore, a simple tyrosinase-based biosensor was constructed for the measurement of wine polyphenolics. Tyrosinase is a copper containing enzyme that catalyzes the oxidation of phenolic compounds found in fruits and vegetables into quinones. We will report on the analytical response of the biosensor to and provide accurate measurement of concentration and reduction potential of polyphenolics in wines.

IV.P.93

PESTICIDE RESIDUES IN WINE: WHY ARE THEY SO LOW?

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**Keywords:** pesticides, wine, persistence

The matter of pesticide residues in food products receives strong media coverage, and wine is not left out. Even if awareness has occurred and a downward trend is underway, pesticides are used to ensure steady quality and quantity of harvests. Questions arose about their occurrence in wine and their toxicity for consumers. Along the year the vineyard receives several treatments and those closer to the harvest, principally fungal treatments, may leave residues on the grapes. These residues can be more or less transferred to the must and may, for some of them, end up in the wines. Unlike fruits and vegetables there are no MRLs for processed products, so there are MRLs for wine grape but not for wine. As a result, no systematic search for pesticide residues in wines is conducted. Analyses are carried out at the producers' discretion, especially when needed for export, or by consumer associations for media purposes.

More than 100 wines, from conventional viticulture, were screened for the presence of 190 pesticide residues by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). From these analyses less than 50 molecules were detected and most of the time at a concentration below 10 µg/L and even close to the limit of quantification. However, some compounds are detected more frequently and sometimes at higher levels. Research programs were conducted to follow the transfer of active ingredients from grapes to must and to evaluate their persistence during winemaking. The transfer from grape to must and the persistence of the active ingredients on grapes were evaluated according to the treatment schedule. To study the transfer rate of the molecules throughout the winemaking, sampling was done all along the process.

**IV.P.94****LEES ADDITION ON UGNI BLANC WINE DISTILLATE: EFFECTS ON FATTY ACID ETHYL ESTERS CONCENTRATION AND OTHER AROMATIC COMPOUNDS****C. DESCOINS**

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**Keywords:** lees, wine, distillation, fatty acid ethyl esters

Cognac is a PDO spirit produced by a double distillation of wines made from Ugni Blanc grown in Charente (SW of France) and aged in oak barrels for, at least, 2 years. The first distillation can be done by adding lees on the wines or not, depending of the traditions of the Cognac Houses. The sensory profile of the distillate will be thus different, especially due to the presence of fatty acid ethyl esters (FAEE) coming from the lees. This study was done during distillation time (October to 31 of march) to have fresh lees. For each replicate, a large amount of wine with lees was centrifugated to separate the wine from the lees. Increasing amounts of lees were then added to the clarified wine to obtain a calibration curve. Each point was distilled in one pass, in a laboratory scale equipment. The micro-distillates were analyzed by gas chromatography to evaluate the amount of FAEE. SPME GC/MS was used to perform non-targeted volatile compound identification of the micro-distillates to evaluate the aromatic impact of the lees.

IV.P.95

VARIETAL DIFFERENTIATION OF PROBUS, RUMENIKA AND FRANKOVKA RED WINES FROM SERBIA BASED ON THEIR PHENOLIC COMPOSITION

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**Keywords:** phenolic composition, wine, principal component analysis, differentiation.

Various factors such as grape variety, environmental conditions and production technology have effects on the wine composition. Specific phenolic composition of wines produced from grape varieties characteristic for Fruška Gora wine region (northern Serbia), namely Probus, Frankovka and Rumenika, was determined in this study. There is not enough available scientific data regarding the phenolic and aromatic composition of wines from these varieties although they have already shown good vinification potential.

Obtained results showed that among wines from different varieties and vintages, statistically significant differences existed in the content of most analysed phenolics. The application of principal component analysis (PCA) showed that the tested wines can be effectively distinguished based on their phenolic composition. Wines made from Probus variety were characterised by very high anthocyanin content, Frankovka wines by high content of syringic acid while high contents of piceid (resveratrol), catechin and gallic acid were specific for Rumenika variety. The applied statistical method proved to be successful in detecting the grapevine variety authenticity markers and the obtained results can contribute to the improvement of data integration and interoperability at the global level.

**IV.P.96****DETERMINATION OF HIGH SUGAR CONCENTRATIONS IN DISTILLED SPIRITS SAMPLES USING A COMPACT ION CHROMATOGRAPHY SYSTEM****P. DEWSBURY<sup>a</sup>**G. Ellison<sup>a</sup>, T. Christison<sup>b</sup>, J. Rohrer<sup>b</sup>

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This poster will demonstrate the determinations of glucose, fructose, and sucrose in flavoured rum, scotch liqueur and Irish whiskey by high performance anion exchange with pulsed amperometric detection (HPAE-PAD). Typically, samples with g/L concentrations require large dilutions from 1000 -10,000 fold to remain in the linear range of the very sensitive HPAE-PAD technique. However, in these applications, the method is performed using a Thermo Scientific™ Dionex™ Integrion™ RFI™ system equipped with a 0.4 µL internal injection loop and the Thermo Scientific™ High Concentration Carbohydrate Analysis Kit to extend the linearity from low mg/L to g/L concentrations, allowing smaller sample dilutions to be made. Having a reduced number of dilutions not only saves sample preparation time but also minimizes the chance of dilution errors. This gives an improved reporting accuracy and confidence in the results.

IV.P.97

**DETERMINATION OF POLAR PESTICIDES IN GRAPES USING A COMPACT ION CHROMATOGRAPHY SYSTEM COUPLED WITH TANDEM MASS SPECTROMETRY**

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**Keywords:** ion chromatography, mass spectrometry, pesticide analysis, glyphosate, AMPA, grape analysis

Polar pesticides in food and beverages have become a hot topic in recent years. A famous representative of this group is a broad-spectrum systematic herbicide called glyphosate and its metabolite AMPA. Glyphosate was discovered more than 40 years ago and became popular due to its low toxicity in comparison with other herbicides. Widely used on vineyards around the globe, the use of pesticides has resulted in residues found on grapes and in turn in the final wine products, not to mention other food and beverages derived from crops. This has led to much controversy as reported in the media and scrutiny from governing bodies such as the World Health Organization (WHO) and the European Food Safety Authority (EFSA), due to the potential adverse effects of pesticides to health. The demand to test for these compounds is sharply on the rise.

Analysing polar pesticides is challenging, they can have low recovery when using liquid/liquid partition methods based on QuEChERS, and poor retention in reversed-phase liquid chromatography. Whilst these challenges can be overcome somewhat with use of solvents or with derivatisation, matrix effects can still be an issue or the process becomes lengthy and less precise. Ion chromatography (IC) is a technique designed for polar analytes and provides excellent chromatographic resolution in a wide range of matrices. Combining this with the power of a highly selective and sensitive mass spectrometer (MS) has led to the development of an IC-MS/MS method for the direct analysis of a total of 16 compounds including 15 polar pesticides and relevant metabolites: glyphosate and metabolites (AMPA and N-acetyl glyphosate), bialaphos, chlorate, cyanuric acid, ethephon (and HEPA), fosetyl-aluminium (and phosphoric acid), glufosinate, N-acetyl glufosinate, MPPA, maleic hydrazide, N-acetyl AMPA, and perchlorate (classed as a contaminant). Using grapes as the sample source, this method was developed with a run time of 20 minutes and detection limits below those required to meet EU maximum residue limits (MRL).





# **Chemical and Biochemical reactions, including grape and wines microorganisms impact**

V.P.1

## COMPARATIVE STUDY OF WINE COMPOSITION IN PINOT NOIR SPONTANEOUSLY FERMENTED IN VINEYARD AND IN WINERY

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**Keywords:** Phenolics, Pinot Noir, Spontaneous fermentation, Yeast

**Background and Aims:** Spontaneous fermentation is driven by a complex community of indigenous yeasts originating from the vineyard which are considered as part of the terroir. *Saccharomyces cerevisiae* has been reported as the most abundant yeast in winery according to previous studies. Thus, our hypothesis of this study was that spontaneous fermentation in vineyard would be a better expression of terroir and reflection of the vintage.

**Methods and Results:** Pinot Noir grapes from same vineyard have been spontaneously fermented in vineyard and in winery respectively in 2015 and 2016. Compared with winery fermentation, wines fermented in vineyard consistently showed significantly lower level of colour density, total red pigments, total phenolics, and tannin. Wines made in 2016 also showed significantly lower level of colour density, red pigments and total phenolics, but no difference in tannin. Phenolic composition analysis by HPLC showed Pinot Noir fermented in vineyard had significantly lower level of a range of benzoic acids and flavonoids.

**Conclusion:** Pinot Noir fermented in vineyard consistently showed lower level extraction of colour and phenolic substances, which is likely due to the variance in yeast population and dynamics during fermentation.

**Significance of study:** This preliminary study could help us better understand the role of wild yeasts in shaping regional characteristics in wine. Further studies will be conducted on aroma profiling of resultant wines and analysing the changes in yeast population and dynamics during fermentation.

## V.P.2

## EVALUATION OF OXYGEN CONSUMPTION KINETICS OF WINE TREATED BY DIFFERENT ANTIOXIDANT PRODUCTS

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**Keywords:** wine, oxidation, antioxidants, reaction kinetics

Sulphur dioxide as wine additive is used because of its anti-oxidative, antioxidant and antimicrobial properties [1]. In winemaking industry, the addition of sulphites is regulated and a general consensus to reduce the use of sulphur dioxide exists due to its potentially harmful effects toward health. In this context, research has been strongly oriented toward the study of alternatives (chemical compounds and technologies) to reduce the use of SO<sub>2</sub> in wine. In various work, different compounds like oenological tannins, lees or yeast derivative have been proposed as antioxidants products [2-3]. Nevertheless, most of these researches are applied in wine model solution [2-4]. Thus, the aim of this study was to evaluate the antioxidant properties of different products (tannins and yeast derivatives) in comparison with SO<sub>2</sub> by evaluation of oxygen consumption rate in (i) model solution and in (ii) white wine.

At first, results in the model solution indicate that oxygen consumption rate, expressed in mg.L<sup>-1</sup>.days<sup>-1</sup>.g of product<sup>-1</sup>, follows second-order kinetics and depending on product concentrations. In the second step, oxygen consumption rate of each product was tested in white wine that was subjected to three consecutive air saturation cycles. The results show that oxygen consumption rate increases after each cycle for all products studied. Nevertheless, each product presented a specific kinetic and their reaction rate cannot be interpreted by simple kinetic model. Analysis of polyphenols, yellow colour and SO<sub>2</sub> in wine after each cycle don't indicate any statistically significant difference. Analysis by cyclic voltammetry of oxidizable compounds in wine show differences between products and control (wine without antioxidant product) after each cycle. A relationship between oxygen consumption rate and detection of oxidizable compounds in wine was highlighted.

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V.P.3

## EVALUATION OF RADICAL SCAVENGING PROPERTIES OF SULFONATED COMPOUNDS IN MODEL WINE

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**Keywords:** NMR, EPR, wine oxidation, radical chemistry

In recent years, the knowledge about the oxidative stability of wine remains limited. In the production of wine, sulfites are used for its preservation and successful aging, since SO<sub>2</sub> has antioxidant and antimicrobial properties. The predominant form of sulfites at the wine pH is the bisulfite form (HSO<sub>3</sub><sup>-</sup>), which reacts with various types of compounds present in wines, such as ketonic acids, sugars, quinones, carbonyl compounds, etc. The sulfonated adducts produced in this way are considered as part of the bound inactive SO<sub>2</sub> fraction. In the present study, the synthesis of several sulfonated compounds that are possibly formed in wine by the reaction of bisulfite with acetaldehyde, pyruvic acid, glutathione, cysteine and ascorbate was carried out in model wine. Both NMR spectroscopy and liquid chromatography-mass spectrometry (LC-QToF-MS) were used to identify and characterize the synthesized compounds. The yields of the sulfonated products were calculated and the radical scavenging properties of the synthesized compounds were studied by the DPPH method and EPR spin trapping methodology. The experimental results are of particular interest, since they show for the first time that sulfonation reaction products can present radical scavenging properties compared to their parent compounds with no antiradical properties. These results provide insight into the possible impact of sulfonation on wine's oxidative stability, and open new ways for managing SO<sub>2</sub> addition during winemaking.

## V.P.4

**EVALUATION OF THE ANTIOXIDANT EFFECT OF BIOPROTECTION FOR WHITE WINEMAKING PROCESS WITHOUT SULFITES**

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**Keywords:** Sulfites, Bioprotection, Varietal aroma, Sensorial analysis

The production of additive-free foods is currently increasing because exposure to food preservatives (sulfites, benzoic and sorbic acid) may negatively affect human health. Due to existing health concerns derived from the consumption of high level of sulfites ( $\text{SO}_2$ ), consideration should be given to use alternatives to sulfiting agents during winemaking. The use of naturally-occurring antagonistic microorganisms in food to enhance food safety, and extend shelf-life without significantly altering the sensory properties of the product is referred to as bioprotection. In the wine industry, bioprotection products have been commercially developed. Recently, products based on non-*Saccharomyces* and *Saccharomyces* yeasts (pure or mixed cultures) were proposed to the winemakers as an alternative to  $\text{SO}_2$  addition at the very early stages of wine-making to protect the grapes and juice from oxidation. The underlying antioxidant mechanisms associated with bioprotection when applied to the white must vinification are so far poorly reported in the literature. The aim of this study is to assess the bioprotective activity of yeasts as an alternative to  $\text{SO}_2$  for wine vinification and to evaluate the impact on the wine's chemical and sensorial composition.

Experiments at different scales (winery and laboratory) were implemented during different vintages. Three treatments were compared: without  $\text{SO}_2$ , with  $\text{SO}_2$  and bioprotection (mix of *Torulaspora delbrueckii* (Td) and *Metchnikowia pulcherrima* (Mp)). The concentration evolutions of the main compounds involved in oxidation reactions occurring in white musts were monitored throughout the pre-fermentary stages. Furthermore, varietal aroma, such as volatile thiols and as well as odoriferous oxidation markers were monitored in wine after alcoholic fermentation and aging. The impact of bioprotection was equally investigated by different sensory tests carried out on wine after aging. Based on the first results obtained, it appeared that the antioxidant effect of bioprotection was vintage dependent. But that the organoleptic component was not impacted significantly in terms of varietal aromas and aromatic intensity.

V.P.5

## MICROORGANISMS OF ALTERATION AND WINE CONTAMINATION : INNOVATIVE TECHNOLOGY TO MODEL ADHERENCE KINETIC AND OPTIMIZE DESINFECTION

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**Keywords:** microbial adhesion, modelisation, circuit test, desinfection

The diversity of materials and the low cleanability of equipments are the main causes of alteration microorganisms presence on wine contact surfaces. The understanding of bioadhesion phenomenon leads, thanks to a collaborative project to the development of a modelling tool. The integration of a pipe equipped with removable disks in a circuit is a technology who allows to follow the adhesive level of microorganisms population in artificial wine matrix, under controlled dynamic conditions (hydrodynamic parameters).

The device permits to work with different materials (inox, PET, wood,...). *Brettanomyces* yeast is the chosen microorganism. The first objective is to reproduce the dynamic adhesion phenomenon, simulating contaminated wine circulation during transfer.

Bioadhesive power of *Brettanomyces* yeasts is observed, and confirm results obtained previously using static methods : microscopic platform.

Once bioadhesion is modelised, desinfection process can be tested. The first step consists in establishing standard adhesion levels on removable disks positionned in a circuit with contaminated wine circulating. The final aim is to appreciate influence of hydrodynamic parameters on adhesive cells elimination and adhesion phenomenon.

Adhesion characterisation before and after the circulation of the solution allows to evaluate its efficiency. It is possible to classify the influence of main hydrodynamic parameters on microbial adhesion : presence time, temperature, active substance concentration.

Results highlight time of presence importance and concentration importance and the nature of disinfectant solution (peracetic acid, chlorinated alkaline, surfactant) influence on adhesive cells elimination.

The model thus obtained allowed to study hydrodynamic parameters of a cleaning process, and underscore the crucial role of flow rate. It also allows to work on resistant cell's capacity to recontaminate an artificial wine matrix.

These references obtained have conducted to recommendation for cleaning process optimization, especially hydrodynamic parameters adaptation and their application on industrial site.

V.P.6

## OXYGEN CONSUMPTION KINETICS DURING ACCELERATED OXIDATION OF RED WINE

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**Keywords:** red wine, oxidation, sulfite, kinetic

Oxygen is playing a major role in wine aging and conservation. Many chemical oxidation reactions occur but they are difficult to follow due to their slow reaction time and the many reactions products that are formed. A forced oxidation protocol was developed to follow the consumption of oxygen in red wines. After oxygen saturation of the wine at 20°C, the red wine sample was heated at 50°C, 60°C or 75°C in a sealed test tube. The oxygen consumption kinetics was followed by using oxo-luminescence oxygen measurement. The oxygen consumption kinetics observed followed a first order kinetics at all temperatures. The reaction at 75°C was 3 times faster than at 60°C and 30 times faster than at 50°C.

The SO<sub>2</sub> protective role as an antioxygen in red wine was also evaluated. Three different SO<sub>2</sub> concentrations (75mg/L, 50mg/L and 25mg/L) were added to a red wine sample heated at 75°C. The kinetics with and without SO<sub>2</sub> were compared to highlight the protective role of SO<sub>2</sub>. When SO<sub>2</sub> was added to the wine, the oxygen consumption kinetic was faster. This confirmed the antioxygen role of SO<sub>2</sub> in addition to the phenolic compounds of red wines.

V.P.7

**REACTIVITY OF DIFFERENT ENOLOGICAL TANNINS DURING OXIDATION: REACTION RATES, INFLUENCE OF SO<sub>2</sub> AND RELATIONSHIP WITH CHEMICAL COMPOSITION AND ELECTROCHEMICAL PROPERTIES**

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**Keywords:** Tannins, Oxidation, Electrochemistry, Sulfur dioxide

Tannins are among the technologically and sensorially relevant components of red wine. Their reactivity towards oxygen is of primary importance in wine ageing, as it relates to anthocyanins stabilization, mouthfeel properties, and wine longevity. Due to the difficulties associated with achievement of suitable tannin levels and tannin-anthocyanin ratios, commercial preparations of tannins of enological interest are often added during fermentation and/or maturation of red wines. These preparations are poorly characterized and their actual contribution to wine phenolic profile as well to wine oxidative behavior remain to be defined.

In this study we have investigated the behavior of five different commercial tannin preparations derived from either grape, grape seeds, oak (two), or tea in model wine solutions during consumption of known amounts of oxygen. The different products were characterized in terms of total phenolic and total tannin content, as well as content of different flavan-3-ols and gallic acid by HPLC. Oxygen consumption rates (OCRs) of the tannin solutions were measured by chemiluminescence in the presence or absence of SO<sub>2</sub>. Electrochemical profiles of the different solutions before and after oxygen consumption were obtained, and this information was evaluated for the degree of correlation with tannin oxidative behavior.

Although oak-derived tannins were characterized by lower total tannins, they exhibited the highest OCRs, whereas grape tannins had the lowest. Total tannins or total phenolics were poorly correlated with oxygen consumption rate, whereas several electrochemical features were correlated with OCRs ( $r^2=0.91$ ). Presence of SO<sub>2</sub> increased OCRs in a manner that was tannin-dependent, with the greatest impact being observed for tea and grape tannins. HPLC analysis indicated that these tannins were richer in catechol monomers. Grape tannins also appeared more prone to oxidative browning, whereas tea tannins oxidation resulted in greater loss of SO<sub>2</sub>. Certain features of electrochemical spectra of tannins appeared to be associated with the degree of involvement of SO<sub>2</sub> in their oxidative mechanism. This study highlights how the compositional diversity existing among commercial tannins greatly affects their ability to react with oxygen and SO<sub>2</sub>. It also shows the potential of electrochemical analytical methods to unravel certain complex aspects of tannin oxidative response in wine conditions.

V.P.8

## REVISITING THE CHEMICAL DRIVERS OF WINE AROMA AGING POTENTIAL

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**Keywords:** aroma, aging, red wine, terpenes

Aroma potential is among the most important factors contributing to the longevity of high quality wines. Acid driven chemical reactions are at the basis of the expression of aroma potential during wine aging. Among these reactions, hydrolysis of glycosidic precursors is considered central to wine aroma evolution, as several potent aging-related aroma compounds arise from these processes. Accordingly, wines with higher concentrations of glycosidic precursors are expected to have greater aroma potential. However, acid-catalysed degradation of some volatile metabolites arising from hydrolysis of glycosides also takes place during wine aging, so that the actual content of a given volatile compound after a period of aging is given by the balance between acid-driven release and degradation. Depending on the aroma potency of the released compound and of the degradation product, these competing processes could lead to either an increase or a decrease in wine aroma complexity.

We studied the evolution of a reference terpene compound such as linalool during wine aging in a set of different Corvina red wines, monitoring at the same time the evolution of its glycosides and degradation products. There was no correlation between the content of linalool precursor in young wines and the content of free linalool in aged wines. However, net variation of linalool during aging was very well inversely correlated with the free/bound linalool ratio in young wines ( $R^2=0.748$ ), so that higher levels of linalool in aged wines were obtained when young wines had a lower free/bound linalool ratios. In addition, in wines where the ratio was high due to high free linalool (e.g. between 40-60  $\mu\text{g/L}$ ), linalool concentration after aging dropped to less than 10  $\mu\text{g/L}$ , indicating that degradation of the free compound was more important than release from precursor. However, at the same time, concentrations up to 30  $\mu\text{g/L}$  of p-menthane-1,8-diol were found, although this compound was not present in the young wines. High correlation between linalool content in young wines and p-menthane-1,8-diol in aged wines ( $R^2=0.7773$ ) suggested that p-menthane-1,8-diol content after aging depends on initial linalool. The aromatic consequence of these ageing patterns are currently under investigation, in consideration of the fact that, although the aroma impact of p-menthane-1,8-diol is low, this compound can act as precursor of other potent cyclic terpenes such as cineoles.

V.P.9

## VOLATILE CHEMICAL MARKERS OF GEOGRAPHICAL IDENTITY OF CORVINA AND CORVINONE WINES

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**Keywords:** Aroma compounds, Geographical identity, Yeast available nitrogen, Acetate esters

Ability of certain wines to express aroma features that can be related to grape geographical origin is a major objective for producers of quality wines. Expressions such as 'terroir', 'regional identity', 'geographical identity' all refer to this ability to transmit a sense of place, and it has been shown that consumers are prepared to pay extra price for such characteristic. Although there is a considerable amount of research concerning the agronomic and viticultural factors characterizing certain supposedly unique grape growing sites, less information is available on how geographical identity is expressed at the level of wines, in particular in terms of relevant aroma chemicals. This is of primary importance for wines from 'neutral' grapes, where the majority of key aroma compounds are present in grapes either in the form of odorless precursors or other putative forms that need complex transformation to generate the actual aroma compounds.

In this study, the volatile composition of Corvina and Corvinone wines from the Valpolicella area, a long established winemaking region famous for red wines such as Amarone, has been characterized. The wines were produced using grapes collected from various vineyards representing different existing 'terroirs', and vinified using the same protocol. The aim was to evaluate which volatile compounds were able to better discriminate geographical origin, providing a basis for improving our understanding of the key factors associated with expression of wine geographical identity. The results obtained indicated that, contrary to the assumption that geographic identity can be observed at the level of grape aroma profile, fermentation turned out to be a crucial step in expressing geographical identity. First, most of the grape-derived volatile compounds that turned out to discriminate wines according to geographical origin, including linalool and other terpenoids, damascenone, vanillates, were scarcely present in the grapes but were mostly released during fermentation. Second, a major component of geographical identity appeared to be related to compounds directly produced by the yeast during fermentation, in particular acetate esters. Analysis of fermentation-relevant grape compositional parameters revealed that grape assimilable nitrogen and in particular primary amino acids were strongly correlated to wine ester content, confirming that this parameter is a central component of geographical identity expression.

V.P.10

## IMPACT OF MALOLACTIC FERMENTATION ON FRUITY AROMA PERCEPTION IN RED WINES MADE FROM MUSTS WITH DIFFERENT ASSIMILABLE NITROGEN LEVELS. EFFECT ON SUBSTITUTED ESTER AND ACID FORMATION.

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**Keywords:** substituted esters and acids, fruity aroma, lactic acid bacteria, must yeast-assimilable nitrogen

Several studies have demonstrated the particular chemical behavior of substituted esters and their sensory impact on modulating fruity expression in red wines, even when these compounds are present at concentrations below their olfactory threshold [1]. Hence, a better knowledge of their origin and the parameters that influence their accumulation is essential for managing fruity aroma and better understand their importance.

Several studies have demonstrated that lactic acid bacteria metabolism [2] and nitrogen composition of the must [3] may strongly influence the levels of a range of volatile compounds, including esters and acids.

Our research followed musts with different nitrogen concentrations through the entire winemaking process to investigate the role of malolactic fermentation (MLF) on the formation of substituted esters and the corresponding acids, considering, where applicable, their various enantiomeric forms. In microvinification experiments, a Merlot must was fermented with an initial yeast-assimilable nitrogen (YAN) content of 111 mg/L, or supplemented up to 165 and 220 mg/L. Two lactic acid bacteria strains were used for MLF.

Must YAN supplementation led to a significant increase of the production of substituted esters of short- and alkyl-substituted fatty acids during alcoholic fermentation (AF) and of hydroxyesters during MLF. Must YAN did not affect substituted acids formation during AF. After MLF, short- and alkyl-substituted fatty acids levels increased in wines made from musts with the highest nitrogen content, whereas concentrations of hydroxycarboxylic acids increased independently of the must YAN content, highlighting the important role of MLF itself. Moreover, sensory profiles revealed a significant increase in black-berry- and jammy-fruit aromas during MLF. A strong positive correlation was observed between these sensory modifications and the production of substituted esters after must nitrogen supplementation and MLF. Finally, aromatic reconstitutions demonstrated that variations in the concentrations of substituted esters during MLF impacted the fruity aroma of red wines.

This work opens up also new investigation perspectives on esters formation pathways, especially those related to hydroxycarboxylic acids as new origin seem to have been highlighted with this study

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V.P.11

**GREEN METHODS TO EXTRACT THIOL PRECURSORS FROM GRAPE MARC YIELD VOLATILE THIOLS WHEN FERMENTED IN SYNTHETIC GRAPE MEDIUM**

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**Keywords:** grape marc, wine thiols, green chemistry, thiol precursors

Grape marc is a major waste product generated during wine production. Because grape marc has a high lignin content and low pH, it is slow to degrade, leading to industrial stockpiling and environmentally hazardous greenhouse gas emissions and nitrate leaching into groundwater. Hence, solutions to ameliorate the issues resulting from this winery waste product are necessary and one avenue we have explored the transformation of grape marc into a value-added product.

Sauvignon blanc (SB) wines, particularly those from Marlborough, New Zealand, are renowned for their high concentrations of volatile thiol compounds, imparting tropical, passionfruit and grapefruit characters to wine. It has been shown that the cysteinylated and glutathionylated precursors of key SB varietal thiol, 3-sulfanylhexan-1-ol (3SH), are located in the grape skins. Therefore, we have developed a completely green method, using only water, to extract the cys-3SH and glut-3SH thiol precursors from SB grape marc.

Quantitation of the vacuum-dried crude extract dissolved in model wine, using liquid chromatography tandem mass spectrometry (LC-MS/MS), showed that the extract contained 1.9 µg cys-3SH and 11.46 µg glut-3SH per 1 g grape marc. Addition of low (3 g) and high (12 g) concentrations of grape marc extract to synthetic grape medium, inoculated with a commercial wine yeast, yielded appreciable concentrations of 3SH in the finished wines (average of 216 ng/L for the low addition and 1244 ng/L for the high addition). Interestingly, the grape marc extract also appeared to accelerate the fermentation rate of the yeast rather than cause any inhibition or toxicity.

This work has successfully developed a method which concurrently reduces the negative impact of grape marc on the environment and provides winemakers with a potential tool for increasing the volatile thiol concentrations in the wine.

V.P.12

## NEW INSIGHTS IN THE ORGANOLEPTIC AND PHYSIOLOGICAL IMPACT OF *SACCHAROMYCES CEREVISIAE* ESTERASE, AN INTEGRATIVE STUDY DURING THE ALCOHOLIC FERMENTATION OF RED WINES

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**Keywords :** Wine aroma, *Saccharomyces cerevisiae*, Fermentation Functional genetics

Esters represent certainly one of the most studied families of volatile compounds since they impact the organoleptic properties of many beverages including wine. They can be classified according to the chemical nature of their precursors. Linear Fatty Acids Ethyl Esters (FAEE) differ from Higher Alcohols Acetates (AHA). A third group, Substituted Ethyl Esters (SE) contributes particularly to red- and blackberry-fruit notes of red wines.

The biosynthesis of linear esters has been investigated at the enzymatic and genetic level. In this work, we confirmed their contribution in the fruity perception in young red wines by achieving gene deletion coupled with chemical and sensorial analyses in Merlot and Tempranillo wines. We confirmed that two pairs of paralogues (*ATF1*, *ATF2*) and (*EEB1* and *EHT1*) are involved in AHA and FAEE biosynthesis, respectively. However, their deletion does not impact the production of Substituted Esters. This third class of esters and their corresponding acid precursors are in contrast affected by the deletion of another pair of genes encoding for mono-acyl lipases (*MGL2* and *YJU3*). These findings open new avenues for understanding the biosynthesis of this impacting class of aromas. Surprisingly the deletion of all these esterase activities triggered drastic genetic incompatibilities. A RNA-seq analysis comparing a wildtype yeast strain and a quadruple-mutant (*ATF1*, *ATF2*, *EEB1* and *EHT1*) shows a drastic patterning of gene expression with up to 1500 genes involved in chromatin organization, RNA transcription and cell division, suggesting an unsuspected regulatory role of ester metabolism.



**Analytical developments  
from grape to wine,  
spirits : omics,  
chemometrics approaches...**

## VI.P.1

# NEW CLUES ABOUT WHITE WINE OXIDATION: CORRELATION BETWEEN VOLATILES AND NON-VOLATILE COMPOUNDS AN INTER-OMIC APPROACH

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**Keywords:** inter-mic, oxidation, volatiles, non-volatiles

Wine oxidation has been a subject of study since decades. The role of specific volatiles on wine aging aroma highlighted the Strecker aldehydes such as phenylacetaldehyde and methional as "key-odorants" regarding oxidation faults.

Several "routes of formation" have been proposed for their increase, namely: oxidation of phenolic compounds in the presence of metals for the formation of o-quinones; the reaction of  $\alpha$ -dicarbonyls with amino acids; and the hydrolysis of adducts between sulphur dioxide and aldehydes formed during fermentation.

Nevertheless, the same factors can affect more than one route rendering the control of the process during wine production extremely challenging.

In that context this study has been divided in two steps : (i) firstly studies in model solutions were performed to better understand the impact of single and interaction between effects such as: metals, oxygen, phenolics concentration and the presence of sugars, which allow us to understand that glucose addition reduces the concentration of phenylacetaldehyde as well as the o-quinone, and the important impact of iron and copper in the phenolic oxidation.

Secondly data-fusion has been proposed as a valuable technique which let us understand interaction between several groups of compounds. New clues about clusterization of volatiles and non-volatile compounds brings new information regarding the interaction between mechanisms and new compounds were identified in particular: SO<sub>2</sub>-phenolics reactions, phenolics-phenolics , phenolics-aldehydes, between others.

## VI.P.2

**BERRY AND WINE METABOLOME PLASTICITY OF A COMMON SCION ON DIFFERENT ROOT SYSTEMS WHILE UNDER VARIED IRRIGATION REGIMES.****Mani AWALE**

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**Keywords:** Rootstock, Grape-derived volatiles, Irrigation, Untargeted metabolomics

The role rootstocks play on mediating environmental stresses on *Vitis vinifera* grapevines have been well studied, however, the effect of rootstocks on the phylloxera tolerant hybrid grapevines and the quality of fruit have not been fully explored. We investigated the plasticity of three commercial rootstocks and own-rooted vines, under different irrigation regimes on wine and berry volatiles in an experimental vineyard in Mount Vernon, MO. The trial contained own rooted Chambourcin as well as three rootstocks (1103P, 3309C and SO<sub>4</sub>). Three levels of irrigation (full compensation of evapotranspiration (ET), 50% of ET and unirrigated) were implemented. We sampled berries at veraison and harvest in 2017 and 2018 as well as made wines on a per vine basis. Samples were analyzed by SPME-GCMS/MS initially using a metabolomics approach followed by confirmation of compound differences using authentic standards. An untargeted approach is more inclusive and better suited to understanding total plant response especially in under studied varieties like Chambourcin. We observed differences in free and bound volatiles in grapes as well as wine volatiles due to irrigation and rootstock treatments as well as an interaction between these variables. Underlying these population differences were a large number of feature differences (m/z by time). For instance, we found 352 significant features in harvest 2018 berry samples across treatments for free volatiles and 520 features that are defining the differences between 1103P and own-rooted berries independent of irrigation (as determined by XCMS software). Feature differences were then used to identify metabolites. Among our findings were that a large number of esters and C13 norisoprenoids such as  $\beta$ -damascenone, were significantly different between own-rooted and 1103P vines regardless of irrigation treatment. However, there were also identifiable differences within the same rootstock, such as in 1103P, where 795 features different between irrigation regimes informed our targeted analysis that were used to identify volatile compounds responsible for those features including TDN, Ionone and Ethyl nonanoate among others. Additionally, the quantitative data from the 2017 wine showed that many important volatile compounds including  $\beta$ -linalool,  $\beta$ -ionone, TDN and  $\beta$ -damascenone are impacted by the irrigation levels as well as the interaction between the rootstocks and the irrigation, defining up to 30% of variance found.

### VI.P.3

## THE RELATIONSHIP BETWEEN ENZYME TREATMENT AND POLYPHENOL/POLYSACCHARIDE EXTRACTION IN WINEMAKING, AND SUBSEQUENT SENSORY EFFECTS IN CABERNET SAUVIGNON WINES

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**Keywords:** polysaccharides, sensory analysis, glycomics, enzymes

Recent work on Cabernet Sauvignon has contributed to a more detailed understanding of the grape cell wall deconstruction process from crushing onwards through a standard red winemaking process<sup>1</sup>. Current research on glycomics in red winemaking suggest that polysaccharides are important sensory impact molecules<sup>2, 3</sup>. We have used glycan-array technology (Comprehensive Microarray Polymer Profiling – CoMPP) as an analytical tool to determine comparative levels of polysaccharides derived from cell walls in red wines. Our experimental system harvests Cabernet Sauvignon grapes at three different ripeness levels and makes wine both with and without enzyme treatment. Initial data shows enzyme addition causes a decrease in soluble polysaccharide content in finished red wines, but an increase in polymeric pigments and tannins. Sensory testing conducted established a relationship between perceived bitterness and astringency and polysaccharide content of red wines. The capacity of enzymes to modulate bitterness and astringency in a wine system via differing polysaccharide extraction will be highlighted and discussed.

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## VI.P.4

## SPATIAL AND TEMPORAL BIODIVERSITY OF GRAPE VARIETIES: THE CASE STUDY OF THE PORTUGUESE BAIRRADA APPELLATION

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**Keywords:** Biodiversity, Grapes, Volatile composition, Component and Specific Weight Analysis

Sustainable viticulture and winemaking continue to represent huge challenges, where a better knowledge about the functional role of biodiversity in the vineyard and wine ecosystems is required. Particular attention should be devoted to the spatial and temporal interactions between autochthonous varieties and climate and vineyard conditions (such as soil type, orientation of the lines, age of the vine, density of planting, harvesting practices, among others). Taking advantages of chemometric tools, this research aims to provide advances to examine interactions between climatic conditions, vineyard ecosystem and a set of white and red varieties. Thus, five varieties (Arinto, Cercial, Bical, Maria Gomes, and Baga *Vitis vinifera* L.), from the Portuguese Bairrada Appellation, were selected as case study. For each variety, grapes from at least two different ecosystems were collected during two consecutive harvests (2017 and 2018). For each variety and vineyard, physical-chemical data from grapes (titratable acidity, pH and sugar content, used to estimate the technological maturity state, and free and glycosidically-potential aroma compounds) and wines (free and glycosidically-potential aroma compounds) were combined with edaphoclimatic data. Relationship between these three data sets and wine quality were studied using Component and Specific Weight Analysis (ComDim). Models for the prediction of grape potential for particular types of wines were developed using PLS regression. The results unveiled the high biodiversity of the Bairrada Appellation varieties. In addition, to the underlying variability of each vineyard ecosystem, each variety presents a specific pattern, which can be expressed differently in the ecosystems under study. The approach used allowed to hierarchize the weight of the different variables and to estimate the adaptability of the five varieties. This tool has high utility for the management and rational use of endogenous resources.

Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/GUI/00062/2019) and CESAM (UID/AMB/50017/2019) through national funds and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020 and to the Projecto Estratégico de Apoio à Fieira do Vinho na Região Centro (CENTRO-04-3928-FEDER-000001). A.R. is funded by national funds (OE), through FCT.

## VI.P.5

# VALIDATION OF MICROSCALE RED WINE FERMENTATIONS (1KG) ON OENOLOGICAL PARAMETERS, COLOR AND AROMA COMPOUNDS: AN EASY RED WINE FERMENTATION METHOD TO PERFORM MANY EXPERIMENTS ON VINES AND WINES.

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## Introduction

The winemaking process is a key factor for many experiments on vines and wines and its implementation is essential to assess the quality and the characteristics of wines. Usually, winemaking is performed on medium batches of grapes from 50 to 100kg. Microscale fermentations (<1kg) allow to i) carry out more experiments than at pilot scale ii) process low quantities of grapes, especially appropriate in case of plant breeding programs.

In this work, small-lot fermentations (<1kg) were compared to fermentations (<100kg) on oenological parameters, color and aroma compounds on five raw materials of red varieties at two stages of maturity in triplicate.

## Materials & Methods

Raw materials for this study were grapes from *Vitis Vinifera* var. Grenache (2), Syrah, Merlot and Cabernet Sauvignon grown in Southern France. Each variety was harvested at two stages of maturity.

For each modality in triplicate, around one hundred kilograms of grapes were destemmed, crushed and distributed into 100 l stainless steel tanks. 900 grams of the destemmed grapes were sampled and kneaded by a lab blender (Jumbomix, Interscience). The small-lot fermentations were performed from an adaptation of the AWRI method (Damberg *et al.* 2010) using "French Press" coffee plunger.

Measurements of oenological parameters, of extraction and color indices (anthocyanins, total polyphenol indice (TPI), color intensity (CI)) and of the major volatile compounds (thiols, esters and alcohols) were carried out on wines at the end of alcoholic fermentation.

## Results & Discussion

### *Oenological parameters*

The low volatile acidity of all the wines showed a good progress of the fermentations. No effect of scale was found on total acidity, pH and alcohol.

Extraction, color indices, volatile compounds

This study showed that anthocyanins, TPI, CI, the major volatile compounds (thiols, esters and alcohols) of the wines from fermentations (<1kg) were correlated with wines of 100 kg scale. Furthermore, wines performed from microscale reached higher values for those indices. This was due to a better extraction with submerged cap maintained by the plunger during the fermentation.

A comparative ranking of the modalities between the two scales were done on all the measurements. This similar ranking underlines the performance of the microscale fermentations (<1kg) showing that this method can be used to integrate the potential of winemaking at the earliest stages of plant breeding.

## VI.P.6

## A MULTIVARIATE APPROACH TO EVALUATING THE CHEMICAL AND SENSORIAL EVOLUTION OF SOUTH AFRICAN SAUVIGNON AND CHENIN BLANC WINES UNDER DIFFERENT BOTTLE STORAGE CONDITIONS

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**Keywords:** Pivot Profile, Storage temperature, aroma stability, Regression vectors

Volatile compounds contribute to the aroma profile of a wine. Wine composition is susceptible to change due to chemical processes such as oxidation resulting from high temperatures and oxygen exposure which may occur during storage and transportation. As a result, changes in sensory attributes may also occur, altering the sensory profile of wine. When it comes to subtle differences between samples, classical univariate analysis that looks at the deviations may overlook the inherent relationships between groups of samples or overall variations due to changes in chemical composition. In this study, changes in South African Sauvignon Blanc and Chenin Blanc aroma profile on the one hand, and in volatile and antioxidant composition on the other hand were investigated using a multivariate approach.

Bottled, unwooded wines of both cultivars from six wineries were stored under different temperatures (room temperature, 15°C, and 25°C) and durations (3 and 9 months). This experimental design resulted in twelve sample sets (two cultivars x six wineries), each containing seven samples (time 0/T0 + 2 storage times x 3 temperature conditions). Chemical composition was determined for each of the 84 wine samples: major volatiles, thiols, SO<sub>2</sub>, GSH, phenolics, and CIE Lab parameters. Pivot Profile® was used to evaluate the change in aroma profile of each sample set. From the resulting chemistry and sensory results, multivariate regression analysis in the form of regression vector (RV) coefficients were used to assess **the correlations in the evolutions of the wines relative to the control, regardless of the original wine profile** (chemical and sensory). A combination of higher temperatures over prolonged periods resulted in the evolution of the aroma profile of the wines from fruity to toasted aroma attributes. The chemical evolution of the wine was highlighted by changes in colour density and hue with increased absorbance at 420 nm (susceptibility to browning).

This is the first time when a data set was used to map and correlate the changes (evolution) in wine samples, rather than simply describe the samples at each stage.

## VI.P.7

# NEW STRATEGY FOR THE STUDY OF GRAPE AROMA POTENTIAL BASED ON THE ACCELERATED HYDROLYSIS OF RECONSTITUTED PHENOLIC AND AROMATIC FRACTIONS (PAFs)

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**Keywords:** aroma precursors, grape quality, accelerated hydrolysis, varietal aroma

Grape aroma potential is one of the key parameters determining the quality of winemaking grapes. However, methods for their evaluation are complicated and do not provide satisfactory data. The direct analysis is almost impossible due to the chemical complexity of the precursor fraction, and methods using enzymatic or acid hydrolysis produce often poor results. In the present paper, a new strategy consisting in the accelerated hydrolysis in strict anoxic conditions of reconstituted phenolic and aromatic fractions (PAFs) extracted from grapes is developed. The scheme of the method is outlined below.

Grapes are crushed in the presence of ethanol to prevent fermentation and to accelerate extraction. After 7 days at 13°C, the ethanolic must (mistela) is pressed, filtered and stored at 5°C. Grape polyphenols and neutral aroma precursors are further extracted using large capacity C18 sorbents. Different parameters affecting extraction were optimized, including the presence of ethanol, type of sorbent and breakthrough and elution volumes. In the final optimized procedure, the extracts contain more than 85% of the total phenolics present in the ethanolic must and all neutral aroma precursors.

The so obtained ethanolic extracts (polyphenolic and aromatic fractions or PAFs) are further reconstituted by diluting with water and tartaric acid to form a reconstituted wine (r-PAFs) which is subjected to accelerated aging. Aging conditions (anoxic/non-anoxic, temperature, time, presence of sugar), including also the possibility of direct aging of unextracted mistela, were also studied, looking for procedures able to produce aged samples (ar-PAFs) containing wine-related high quality and intense aromas in a reasonable time.

Anoxic conditions and the presence of polyphenols are two essential conditions in order to preserve aroma quality. Sugar has to be removed to avoid the development of syrupy flavors which mask the varietal aroma developed. Additionally, the presence of sugar seems to induce the development of kerosene notes attributed to TDN. At 45°, at least 7 weeks are required to obtain a satisfactory aroma expression from high quality grapes, while at 75°C 24 hours are sufficient. Although DMS precursors are not present in the PAFs, the ar-PAFs obtained following the optimized procedure show an interesting range of wine-related intense aromas, suggesting that the strategy may be useful a tool for the study of the aroma potential of winemaking grapes.

*Acknowledgment.* Work funded by the Spanish MCIU AGL2017-87373-C3-1R. Y.A. and LAAE acknowledge the Diputación General de Aragón for a predoctoral fellowship, as well as the European Social Fund.

## VI.P.8

STILBENE PROFILES OF CANES FROM WILD *VITIS* SPECIES BY UHPLC-QqQGrégoire LOUPIT<sup>1</sup>Céline Franc<sup>1</sup>, Tristan Richard<sup>1</sup>, Sarah Cookson<sup>2</sup>, Josep Valls Fonayet<sup>1</sup>

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**Keywords:** Stilbenes, Grapevine cane, Quantification, *Vitis*

Grapevine canes contain high levels of phenolic compounds such as stilbenes. These bioactive compounds have many interests in viticulture since they can act as alternative to chemicals and they can also be involved in the compatibility of grafting. In this project, we developed an HPLC chromatography coupled with a Triple quadrupole mass spectrometer (QqQ) in order to separate and quantify 23 stilbenes from 14 different wild *Vitis* species.

The canes were pruned in winter, cut and ground to make powder. Extraction of the polyphenols was optimized and final conditions were extraction with methanol using ultrasound for 10 minutes followed by centrifugation for 5 minutes at 13,000 rpm.

The HPLC-QqQ conditions were optimized for all the compounds in the positive and negative mode by infusion in the mass spectrometer, and a quantifier and at least one qualifier were selected for each compound. Final conditions allowed for the separation of the analytes on a Poroshell C18 column under 20 minutes. The quantification was established with external standards for each compound, except for cis-stilbenes, which were quantified as trans-stilbenes, and stilbene glucoside oligomers, which were quantified as the non glucosylated oligomers.

The results showed that the total stilbene concentration was higher in *V. thumbergii* and *V. rupestris* (higher than 3 g/kg FW), while *V. berlandieri* and *V. davidii* showed the lowest levels of stilbenes (circa 0.5 g/kg FW). Concerning the stilbene profiles, they were different among species. The dimer E-ε-viniferin was found to be the main stilbene in almost all canes (range between 31 and 89% of the total stilbenes), but some varieties such as *V. rubra* contained remarkable amounts of tetramers (up to 55% of the total stilbenes).

These results can assist on the selection of the most suitable species to obtain stilbenes-enriched extracts

## VI.P.9

## SYNTHESIS OF BIOACTIVE COMPOUNDS (HYDROXYTYROSOL, MELATONIN AND OTHERS DERIVATIVES OF TRYPTOPHAN) DURING ALCOHOLIC FERMENTATION.

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Hydroxytyrosol (3,4-Dihydroxyphenylethanol) is a phenolic compound that could be formed from a degradation or transformation from other polyphenolic structures present in wines (i.e. anthocyanins [1]). Likewise, it is formed from oleuropein degradation in olive oil [2]. The mean intake of HT from a moderate consumption of wine (100–200 mL/day) ranges between 0.15–30 mg/day [3].

HT possesses antioxidant, cardioprotective, neuroprotective, antiinflammatory, antimicrobial, antidiabetic and antiatherogenic properties [4] and it trespass the BBB [5]. Melatonin (N-acetyl-5-methoxytryptamine) is present in wines and it has a role in the winemaking process, besides it is present in olive oil and beer. The concentrations varying between 5 µg/g or mL to 230 µg/g or mL [6,7]. Melatonin has received a great deal of attention as a potential bioactive compound to prevent cardiovascular diseases as well as, some types of cancer. Indeed, it has proved to significantly inhibit VEGF-induced VEGFR-2 activation in human umbilical [8]. Moreover, Melatonin shows an inhibitory and destabilizing effect on amyloid-β peptide fibril formation as recently reported showing neuroprotective properties [9]. This communication presents the results of the synthesis capacity of these bioactive compounds by different yeast strains under fermentation conditions. Hydroxytyrosol and Melatonin were determined both in the intracellular and extracellular medium. Hydroxytyrosol was identified in three strains during alcoholic fermentation in different varieties of grapes and synthetic must by means of a developed and validated UHPLC–HRMS method. The concentrations found in the intracellular medium are in the range between 8.6 and 106.2 ng mL<sup>-1</sup> and in the extracellular medium are between 235.0 and 400.0 ng mL<sup>-1</sup> [10]. Melatonin was identified in seven commercial strains during alcoholic fermentation in synthetic must using a UHPLC–HRMS system (Gexactive). The average concentration found are in the range from 0.60 to 1.0 ng mL<sup>-1</sup> [11].

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## VI.P.10

## TARGETED SCREENING OF 35 ENDOCRINE DISRUPTORS RELEASED FROM PLASTIC BASED FOOD CONTACT MATERIALS

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Food contact materials are all materials intended to come into contact with food. Modern food packaging materials are designed to fulfil multiple purposes including the protection and the preservation during transportation and storage. Concerning food safety, one of the major considerations of manufacturers must be the migration of harmful chemical compounds from packaging materials to food, as they could adversely affect consumer health. In this context, strict national and international regulations, applicable to all materials which come in direct contact with food, have been established. The production and use of plastic packaging materials have increased in the wine industry during the last decades, comprising an indispensable part of wine manufacturing. During the polymerization process of plastic, some molecules are generally added to increase elasticity, flexibility, color, resistance of durability of materials. According to the plastic formed, these additives, which some are suspected to be endocrine disruptors, can be released into the food and endanger human health.

In order to evaluate this release and the potential exposition to toxic effect, a generic UHPLC-MS/MS method was developed to map out different classes of compounds without discriminating the material nature. About 35 compounds were studied including different classes of substances as alkylphenols, amines, bisphenols, BADGES derivatives, benzotriazoles and phthalates. These molecules are all suspected to have endocrine disruption properties.

The first step of the development was the optimization of each parameter of the mass spectrometer and the liquid chromatograph: the ionization mode, the transitions choice for each compound, the mobile phase composition. Two transitions were used for each compound, the most selective and with higher abundance for quantification and the second one, for confirmation in MRM mode. Bisphenols were ionized in a negative mode with the formation of  $[M-H]^-$  precursors whereas phthalates, alkylphenols, amines and benzotriazoles were ionized in a positive mode with the formation of  $[M+H]^+$  precursors. BADGES were ionized in a positive mode with the formation of  $[M+NH_4]^+$  precursors. Two kinds of ionization sources were tested: ESI Jetstream and Atmospheric Pressure Chemical Ionization. ESI gave better overall intensities for all compounds tested and was finally chosen. The separation of these different molecules was a challenging task due to their different structures and chemical properties ( $\log P$  ranging from -0.4 to 10). In this way, five different stationary phases were evaluated, and best results were obtained using a core-shell polar based support, which was able to achieve baseline separation of two pairs of isobaric phthalates possessing the same transitions. Moreover, final conditions included a methanol/water mobile phase delivered at a flow rate of 0.7 mL/min and a total run time of 15 min.

The method was fully validated using official simulant consisting of a hydro-alcoholic solution of 20% ethanol kept at 40°C during 10 days. Exactitude profiles were constructed for all compounds tested in a defined concentration range allowing very low concentration to be achieved (ppb level for most toxic compounds). As a first application of the developed method, few plastic films used by the wine industry have been tested. An official simulant solution (hydroalcoholic solution 20 % v/v) was used to simulate the wine contact. A release of 350 ppb of 4-nonylphenols from the packaging in the simulant solution was observed after only one day of contact. 4-nonylphenols is a degradation product of an antioxidant used in polyethylene manufacturing and is considered as an endocrine disruptor. These results demonstrated the need to possess powerful analytical tools to monitor such contamination and avoid such exposition through foodstuffs absorption.

VI.P.11

## UNRAVELLING REGIONAL TYPICALITY OF AUSTRALIAN PREMIUM SHIRAZ THROUGH AN UNTARGETED METABOLOMICS APPROACH

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**Keywords:** chemometrics, feature selection, regionality, volatile compounds

The association of agricultural products to a provenance or specific region (terroir) that imparts a typical and unique sensorial profile is an important concept for providers of high value products, especially in the wine industry. Shiraz is one of the most widely planted grape varieties in Australia, considered as an icon for a number of wine regions. The current project seeks to characterise the complex wine volatile composition through an untargeted metabolomics approach, to uncover the underlying patterns that distinguish premium Shiraz wines from different regions.

Twenty two wines were chosen from 6 different regions across Australia. Volatile fractions were extracted in triplicate, using a solid phase extraction protocol. The extracts were analysed with a gas chromatography quadrupole time-of-flight mass spectrometry (GC-qTOF MS) operated in the scan mode. The collected data files were processed with a suite of open-source R packages, including xcms and metaMS. Over 10,000 features (ion - retention time pairs) were extracted and important features were identified using statistical analyses. These features were then grouped into pseudo-spectra, some of which could be identified by comparing with spectra of pure standards measured with the same system. Preliminary results showed a clear separation of Hunter Valley from other Australian regions. Furthermore, some separation based on producer was also evident within regions. Combining with sensory evaluation and climate data, results from current project will help unravel the underlying factors that define the premium Shiraz wines from different Australian regions.

## VI.P.12

## USE OF NEW SPME-ARROW-GC-MS/MS FOR THE ANALYSIS OF WINE CONTAMINANTS

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**Keywords:** SPME-arrow, pesticides, haloanisoles, halophenols

New developments in Solid-Phase MicroExtraction (SPME) led to the release of a new tool called SPME-arrow consisting in a thicker coating increasing analyte capacity and terminated with an arrow to facilitate the piercing of septa and to better protect the coating phase when retracted into the needle. Coupling the SPME-arrow to a gas chromatograph with tandem mass spectrometry detection (GC-MS/MS) provides a highly selective and sensitive instrument fully automated from extraction to detection.

The purpose of this work was to test the capabilities of SPME-arrow-GC-MS/MS in two areas, pesticide residue analysis and joint analysis of haloanisoles and halophenols.

Concerning pesticide, a large number of active ingredients, with various physico-chemical properties, are authorized for vineyard protection. Extraction from food products is generally performed using the QuE-ChERS extraction. However, since the wine is an aqueous liquid matrix, other extraction techniques can be used and, in particular, SPME has been found to be a simple, automated, and effective approach. The expected benefits of SPME-arrow-GC-MS/MS are (1) the lowering of limits of detection (LODs) due to the higher amount of coating, the possibility of working in immersion and the greater selectivity and sensitivity of detection, (2) the time saved in terms of sample preparation due to the reduction of the number of steps and automation. More than 100 active ingredients have been tested in terms of linearity, LOD, repeatability, matrix and memory effect. For a wide range of compounds good linearity and repeatability as well as extremely low LOD were obtained. The only drawback may lie in the memory effect which alters the quantification at ultra-low concentrations for some compounds, with the risk of giving false-positives.

SPME-arrow-GC-MS/MS with derivatisation was used for the simultaneous analysis of haloanisoles (HA) and their precursors, halophenols (HP). These compounds are responsible for cork taint and dusty, mouldy odours, and have an impact on wine quality even at concentrations below their sensory thresholds masking the wine fruity aroma. MS detection was optimised showing that ionisation at 30 eV gives the highest peak area of the quantifying transitions. First results show acceptable LOD for all compounds with a reduced sample preparation time. Optimisation is still in progress but only a slight memory effect could be noticed not impacting the quantification results.

## VI.P.13

## MINT AROMA COMPOUNDS IN RED WINE: DETERMINATION BY A NEW AND SIMPLE METHOD BASED ON AUTOMATED HS-SPME-ARROW AND GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY ANALYSIS

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**Keywords:** mint aromas, red wine, SPME Arrow, method validation

The wine ageing bouquet is the expression of high quality fine wines. This quality is linked to the absence of off-flavor, complexity, freshness. Great aged wines keep a refreshing dimension. The olfactory concept of the ageing bouquet of fine red Bordeaux wines has been defined. Among the sensory descriptors minty notes were found to play a role in the definition of this concept [1]. Further chemical and sensory investigations led to identification of several compounds derived from the limonene biotransformation pathway, such as piperitone, p-menthane lactones, carvone, pulegone, potentially responsible for positive olfactory mint aromas [2] [3]. To study the quantification of the pool of limonene-derived mint aroma compounds, a first method was proposed, consisting in a double stage of pre-concentration and extraction of the analytes, by coupling Solid Phase Extraction (SPE) and Stir Bar Sorptive Extraction (SBSE), followed by GC-MS analysis [4]. Although this method allows a reliable determination of the analytes of interest at trace levels, it was complex and very time and wine consuming.

To overcome these limitations, a fast and simple method for assaying limonene-derived mint aroma compounds in red wine has been developed. It couples the new SPME Arrow extraction to the high sensitivity of a Triple Quadrupole GC-MS/MS instrument. First of all, an optimisation step of MS detection was carried out and then, various parameters affecting the extraction efficiency were tested. The highest peak area for each compound was obtained at 30 eV ionization energy. Six different fiber coatings were tested and PDMS-DVB resulted to be the best compromise for the detection of the most limiting compounds.

The extraction conditions by HS-SPME Arrow were optimized for the selected fiber, in order to enhance the overall method sensitivity. The following parameters were optimized by comparing the absolute response of each compound: dilution of the sample (not diluted, 2-fold and 5-fold dilution with ultra-pure water), ionic strength (0, 1.5, 3, 3.5, 4, 4.5 g NaCl), extraction temperature (40, 50, 60 °C), extraction time (40, 60, 80 min), and stirring speed (500, 1000, 1500 rpm). The optimized method was validated and showed good linearity, repeatability, reproducibility, accuracy and the required low detection and quantification limits. The performances of the method were tested on real wine samples and the mean concentrations of the mint compounds were determined in 14 red Bordeaux wines.

With this new simple and fast method, it will be possible to deepen the knowledge on the aging bouquet of red Bordeaux wines.

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**Sensory properties,  
psychophysics,  
experimental economy,  
connections with  
neurosciences**

## VII.P.1

# OAK WOOD VOLATILES IMPACT ON RED WINE FRUITY AROMA PERCEPTION IN VARIOUS MATRICES

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**Keywords:** Red wine, Perceptive interactions, Oak wood, Fruity aroma

Perceptive interactions involved in wines aroma constitute a field where many data have been collected for about 10 years. First works have been performed on red wines produced from traditional Bordeaux varieties *Vitis vinifera* cv Merlot noir and cv Cabernet-Sauvignon. These demonstrated that some ethyl esters and acetates, mainly substituted and present at concentrations below their olfactory threshold, were responsible for at least one part of the specific fruity aroma of these wines.

Further works showed that the perception of these esters "fruity pool", always thanks to particular perceptive interactions, could be modified according to the occurrence of non-fruity compounds leading to synergistic effects as well as suppressing or masking effects.

Oak wood is an invaluable partner for great red wines elaboration. Oak compounds have, indeed, a particular impact. Their presence is known to improve the quality of wines, bringing richness and complexity. Numerous studies have highlighted the role of oak barrel or chips on the aromatic expression of wines, but very little on the impact on their fruity expression. Moreover, most of these studies used principally statistical correlations even if some authors have shown, in synthetic solution, the impact of wood derived compounds on fruity perception, through particular perceptual interactions.

We have developed a wide experimental design to characterize, at different concentrations representing the diversity of cooperage practices, the impact of various volatiles derived from oak wood, on red wines fruity aroma expression, thanks to numerous aromatic reconstitutions both in dilute alcohol solution and in commercial red wine.

New particular perceptive interactions were highlighted and the fruity character enhancing potential of some oak volatiles have been demonstrated. Such an approach allows to imagine how to predict the impact of cooperage practices, including oak origin and toasting, on red wines fruity aromatic expression.

**VII.P.2****SENSORIAL AND CHEMICAL DESCRIPTION OF THE AGEING BOUQUET OF CHAMPAGNE RESERVE WINES.****Nicolas LE MENN**<sup>1,3</sup>Richard Marchal<sup>2</sup>, Dominique Demarville<sup>3</sup>, Gilles de Revel<sup>1</sup>, Stephanie Marchand<sup>1</sup>

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**Keywords:** Ageing bouquet, Conceptual description, Champagne, Aroma

The wine ageing bouquet is one of the most fascinating phenomena in oenology.

It denotes a set of aromas, which, together, form a perceptible equilibrium of pleasant sensations. "Reserve wines" are still wines, stocked by Champagne winemakers and integrated in the blend after several years of ageing mainly in tanks. Some Champagne producers own a large diversity of wines issued from the 3 varieties chardonnay, pinot noir and meunier. This kind of collection constitutes an extraordinary exploration field for scientists who want to investigate the composition of aged wines.

Using a multidisciplinary approach [1], [2], the conceptual and sensorial description of Champagne reserve wines have been done. The ages of wines and the grapes varieties have been considered as variables. Results present two main points. The first one is a convergence of the aroma descriptions of wines produced from the 3 varieties chardonnay, pinot noir and meunier with ageing. The second one is the presence, in the description, of notes already cited in the description of Bordeaux red aged wines[1] and the presence of more specific aromatic notes sometimes in the description of aged Champagne or chardonnay wines [3], [4]. The two "models" red Bordeaux wines and Champagne reserve wine are really different and could be qualified as "opposed" (acidity, pH, vinification strategies, ageing strategies, nitrogen status, presence of wood, phenolic contents, varieties...) however the presence of common notes suggests similar ageing mechanism and similar molecular reality including precursors.

After the conceptual and sensorial description, a molecular one has been started. The importance of some heterocycles in the constitution of the champagne reserve wine bouquet have been explored[5]. The role of dimethylsulfure has been studied and, as for red Bordeaux wines, this compound seems to play a central role in the quality of old wine aroma. Finally, using an untargeted approach two new odorless compounds have been detected and quantified in Champagne wines. The levels are linked to the wines ages.

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**VII.P.3****CELL WALL MANNOPROTEINS FROM YEAST AFFECT SALIVARY PROTEINS-FLAVANOL INTERACTIONS THROUGH DIFFERENT MOLECULAR MECHANISMS**Elvira **MANJÓN**<sup>1</sup>Natércia F. Brás<sup>2</sup>, Ignacio García-Estévez<sup>1</sup>, M. Teresa Escribano-Bailón<sup>1</sup>

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**Keywords:** astringency, mannoproteins, salivary proteins, flavanols

Nowadays, numerous wine industries are introducing some mannoprotein-based products in different stages of red winemaking [1]. Particularly, the addition of commercial yeast mannoproteins (MPs) to wines is a practice leading to modulate harsh astringency. This sensory property mostly results from the interaction between phenolic compounds and salivary proteins (SP). The positive effect of natural yeast MPs on the astringency has generated a growing interest to better understand the molecular mechanisms that allow this modulation. In this work, we have studied the effect of different commercially available MPs on the interaction between flavanols and salivary proteins by using Isothermal Titration Calorimetry (ITC). ITC is a useful technique to characterize the main mechanisms and the forces driving interactions, allowing to obtain the thermodynamic parameters associated with the analysed interactions (affinity constant ( $K_a$ ) and changes in enthalpy ( $\Delta H$ ), Gibbs free energy ( $\Delta G$ ) and entropy ( $-T\Delta S$ ) [2]. In addition, for the purpose of complementing the results obtained, we have analysed the ternary interaction by Molecular Dynamics simulations. MD simulation is a computational method that uses model molecules in order to understand their interactions at an atomistic level and that has been previously used to explain satisfactorily the synergistic effect on astringency elicited by mixtures of flavanols [3]. To perform these experiments, we have selected the procyanidin B2, the peptide IB9-37 and two mannoproteins with different saccharide/amino acid ratio.

Our results indicate MP-SP-flavanol interaction takes place especially through hydrophobic interactions and hydrogen bonds. Moreover, we have been able to observe that the compositional characteristics of the MP used determine the strength of the interactions and, therefore, the capability of MP to affect flavanol-SP aggregation, which would modulate the astringency through formation of soluble ternary aggregates or preventing the flavanol-SP interaction by a competitive mechanism.

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## VII.P.4

# CHARACTERISING THE AROMA PROFILE OF PINOT BLANC: A CASE STUDY OF THE SOUTH TYROL WINE REGION

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**Keywords:** Pinot blanc, Terroir, Wine aroma, South Tyrol

**Context and purpose of the study** - Among the different factors that can be involved in the development of the aromatic profile of a wine, great importance is ascribed to the rough material undergoing the fermentation process; especially in the case of neutral grape varieties characterised by no dominant flavours. On this basis, the widely known cv. Pinot blanc represents an identifying grape variety for the viticultural region of South Tyrol (north-Italy). It was aimed by this study to deepen the influence of different growing sites' altitude and harvest time on the aroma expressiveness developed by the wines under investigation (year 2017), in order to describe an overall sensory pattern of the typical Pinot blanc from South Tyrol.

**Material and methods** - Eight vineyards of cv. Pinot blanc distributed along the Adige Valley were selected to obtain a representative pool of four different growing areas spread in the region. The sites were located at different altitudes, ranging from 230 to 700 m.a.s.l. From each vineyard, the grapes were picked at two moments, aiming to target different ripening levels. The juices extracted after crushing and pressing were then processed following a standard vinification protocol. After conservation and stabilisation, the wines were subject to sensory analysis by a trained panel of tasters. Data of sensory attributes were collected out of three tasting sessions and elaborated to assess if any of the differences displayed by the sensory panel could be significantly influenced by the considered factors "altitude" and "harvest time".

**Results** - All wines presented different expressiveness depending on the belonging altitude and their picking time. According to the panel, more complexity and general appreciation were given to the wines corresponding to higher altitude and delayed picking. However, despite sourness, the only aroma influenced by the two factors was that of banana, whilst the intensity of peach displayed significant difference with regards to altitude only. These results confirm the importance of a good fermentation management, responsible for the aromatic substances quality in this case attributed to the esters production. In conclusion, altitude and harvest time may impact effectively on the ripening course of the grape fruit. Therefore, these two factors could represent valid tools for the decision-making process of the viticulturist that seeks for more expressiveness in wines processed out of a neutral grape variety such as Pinot blanc.

## VII.P.5

### CHEMICAL AND SENSORY DRIVERS FOR WINE QUALITY

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**Keywords:** quality drivers, wine quality, sensory/chemistry data fusion

Sensory properties \*

Wine quality is a concept difficult to define and therefore often avoided in scientific works. Nevertheless, wines are often valued for their quality as perceived by professionals in the industry and every year many prestigious local and international competitions take place.

Our work focuses on two such competitions that take place each year in South Africa, Top 10 Pinotage and Top 10 Chenin Blanc, two of the country's most iconic wines. When the winners are announced, the Top 10 wines and additional lower scoring wines are submitted to sensory and chemical analysis. The sensory evaluation consists of a CATA exercise followed by quality rating on a 20 point scale undertaken by wine industry professionals. This type of evaluation results in the profiling of the wines and also the re-evaluation of their quality scoring in a non-competition setting. Wine fingerprinting by HRMS completes the samples' characterisation using an information-rich chemical approach.

Data obtained from both evaluations is submitted separately to the appropriate statistical analysis (CA and PCA, respectively), from which the standardised deviates for the samples are calculated. The results are used to elucidate the chemical (ions/compounds) and sensory (attributes) drivers for quality in each data set (Pinotage and Chenin Blanc).

The statistical aspects of this type of work can be challenging, but the benefits are two-fold: (i) advancing practical strategies for reliable and rugged sensory/chemistry data fusion, which can be applied in other contexts; and (ii) working towards a possible approach to a more objective "wine quality" concept as defined by the intrinsic and measurable sensory and chemical characteristics of the samples

## VII.P.6

### IMPACT OF LEARNING AND TRAINING ON WINE EXPERTISE

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**Keywords:** sensory abilities, wine tasting, sensory training, sensory learning

Experts are able to conduct both analytical (segmentation) and holistic tasks (wine categorization) involved in evaluating wine quality. Although there is no standard for wine-tasting training, a number of papers have reported on experts' ability to discriminate among and categorize wines, highlighting a process involving perceptual clues and cognitive processing. This work summarizes the influence of different training tasks on tasting and olfactory abilities.

Moreover, recent researches in psychophysics and neurosciences demonstrated the potential functionality of mental imagery. Mental imagery is defined as the creation of a neural representation in the absence of an external stimulus or event and is accessed from memory.

Subjects are not equally able to form olfactory mental images and that this ability is an important part of sensory expertise. This ability may play an important role in wine discrimination and categorization, but is dependent on preliminary perceptual, attentional, and semantic training.

**VII.P.7****NEUROPROTECTIVE EFFECTS OF SOME GRAPEVINE POLYPHENOLS****Alessandra FERRANDINO**

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**Keywords:** brain cell death, anthocyanins, flavonol, flavan-3-ol

The link between wine-consumption and health is complex. Polyphenols (PPs) are, among others, responsible of grape and wine antioxidant capacity (AC) and thus beneficial to health. Although there is a huge body of pre-clinical evidences on the several cellular mechanisms targeted by PPs (resveratrol, in particular), clinical studies have confirmed only few of them. Therefore, information is very confusing and a sound scientific proof of the many different protective properties that, at times, have been attributed to grape extracts and wines is still missing. Besides, the link between wine and "BRAIN health" is further complex as an excessive consumption of alcohol is beyond any doubt detrimental to the development and maturation of the nervous system, and alcoholism/binge drinking are severe socio-economic plagues. The scientific community accepts with some difficulties or even scepticism the idea that wine antioxidants can be effective in neuroprotection, although certain well-demonstrated favourable effects in combating the loss of neurons that follows some inflammatory and/or degenerative processes were ascribed to wine antioxidants. Paradoxically, consumers accept this idea more easily and require wines with low alcohol content but high concentration of PPs.

Some polyphenols, known to be accumulated in grape berry skins, malvidin 3-O-glucoside and peonidin-3-O-glucoside (anthocyanins), quercetin 3-O-glucopyranoside (flavonol), (+)-catechin (flavan-3-ol) and resveratrol (stilbene) were tested in ex vivo conditions by measuring quantitatively the entity of dead cells after their distribution in mouse brain neurons, organotypically grown (Lossi *et al.*, *Mol Neurodegener* 2016 11:34. doi: 10.1186/s13024-016-0101-8).

Results showed that the tested polyphenols displayed an important role in limiting the number of dead cells. In particular, peonidin-3-O-glucoside and (+)-catechin displayed the highest efficacy, followed by resveratrol. Quercetin 3-O-glucopyranoside and malvidin-3-O-glucoside did not significantly limited cell survival. Further experiments are ongoing to test other grapevine polyphenols, known to characterize the polyphenolic profiles of berries (Ferrandino *et al.*, *J Agric Food Chem* 2012 60: 4931-4945) and leaves (Kedrina-Okutan *et al.*, *J. Agric. Food Chem.* 2018, 66: 10977-10990) of some *Vitis vinifera* varieties, with the final goal to individuate possible promising therapeutic molecules for preventing neuron apoptosis.

## VII.P.8

## SENSORY CHARACTERIZATION OF SAUVIGNON WINES

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**Keywords:** Sauvignon wine , Check-All-That-Apply method , Sensory analyses

Worldwide, there is an increasing demand for wines being round, fruit and silky. To meet these consumer wishes, winemakers will have to adapt their practices to obtain products corresponding to the international taste. One of the most popular grape variety for obtaining fruity wines is sauvignon blanc. However, before attempting to identify the levers for obtaining fruity wines of sauvignon, it is important to have a global vision of the wines available on the market. Therefore, the aim of the present work was to describe sensory universe of sauvignon wines, to better characterize this grape variety.

For this purpose, it was chosen to analyze 52 international Sauvignon wines (a majority came from France, but also from New Zealand, Chile, Australia and South Africa). Olfactory sensory characterization by the Check-All-That-Apply method (CATA) was conducted by a 19 trained panel. This method consists in presenting a list of terms in which judges must select terms to describe products. The list was established from a bibliographic work to target the terms that characterize sauvignon wines and more generally white wines. For evaluation, wines were randomly presented to judges and evaluated in black glass in duplicate. Instructions were: « describe the perception that you have of the wine by selecting, from the list, 1 to 3 terms characterizing the principal odor and 0 to 3 terms characterizing the secondary odor ». Then, statistical processing of CATA data was performed with agglomeration hierarchical clustering (AHC) and correspondence analyses (CA) on a contingency table.

The first result was the validation of the list of judgment criteria. Among the 58 descriptors describing olfactory sensations, 54 were selected for their quotient frequency greater than 1 and their significant difference between wines. At the end of the study performed with the CATA method, groups of wines having close sensory properties were obtained. Five groups of wines having different sensory characteristics were highlighted. For these groups, the discriminating variables are: fruity, empyreumatic, amyl-floral, pastry and exotic fruits. The geographic origins of wines partly explain these sensory groups. For example, New Zealand wines are differentiated by the exotic fruit odor and are significantly different from other wines.

Within the "NV<sup>2</sup>" project (that brings together 4 private companies, 1 technical institute and 3 public institutes), the next step will be to correlate these results with chemical analyses of aromas.

## VII.P.9

### THE WINE SERVICE: LAST ACT IN FAVOR OF TASTING

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**Keywords:** wine, carafage, oxygen

Carafage is a common practice used by individuals and wine professionals wishing to provide oxygenation benefits to the organoleptic aspects of wines. The proper procedure for a perfect carafage differs depending on the wine and the sommelier experience, making this practice complex and a sometimes mysterious. There are not many scientific studies about this practice, and its real impact on wine can raise the question about the utility of this procedure. This work is based on an overall inventory of this practice and aims to check if carafage has some impact on wine quality or not.

Firstly, professional sommeliers were asked to describe a general consensual practice for carafage. We thus obtained a broad overview of the different techniques, of the equipment and of the general methodology. It was also asked to indicate the best examples of wines enhanced by carafage: Bordeaux Cabernet Sauvignon and Rhône Valley Syrah were mostly cited. The protocol set-up here is based on sensory analysis of wines selected by sommeliers in order to establish a sensory profile for each wine by rating organoleptic smell and taste descriptors. Each wine underwent different carafage periods before being submitted to the expert panel of the Changins school, in order to evaluate its organoleptic evolution with time. Then we associated the statistical results to the objective sulfite and polyphenol contents to check for a possible correlation. Polyphenol content was evaluated using usual spectrophotometer measurements (IPT and total tannin content) and oxidizable compound content was quantified using the NomaSense PolyScan P200 (Vinventions).

Carafage had a positive influence on the fruity character of the Bordeaux Haut-Médoc 2012, while the Rhône-Valley Syrah 2016 showed a significant negative relationship between the appreciation of the wine by the panelists and the aromatic intensity of the wine smell, thus preferring the "bottle" variant (without carafage). carafage has thus an impact on the organoleptic profile of wines, whether positive or negative. We were able to highlight significant variations of wine properties depending on carafage time. This demonstrates the complexity of this operation when trying to obtain optimal aromatic and sensory enhancements for this unique and delicate product.

## VII.P.10

## PROGUSTO, THE FIRST SMARTPHONE APP FOR PROFESSIONAL TASTING. DEVELOPMENT OF TASTE AND AROMA PICTURES BOOKS TO HELP SENSATION TRANSCRIPTIONS

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**Keywords:** tasting , pictures

All the wine professionals' tasters have been confronted to the problems of recording and of classification of the tasting notes. Some of them use notebooks of wine; others use cardboard that were then classified and/or forgotten; the most modern uses laptops, notepad or smartphones. It is by observing these professional tasters that the idea of working on the construction of a smartphone application adapted to all professionals tasting situations (grapes and wines). Now ProGusto runs on Android and iOS systems in French and English languages.

It accords to the teachings of the Bordeaux oenologist Émile Peynaud<sup>1</sup> who imposed an analytical approach to professional wine tasting. It seemed essential to make this approach accessible to all through a modern tool. On another hand, the description of the wines smells, tastes and other sensorial stimuli is a complex and multifactorial task. It includes odor detection and extraction from the matrix baseline, stimulus recognition and at the end the proposition of an adequate descriptive word. That task, the verbalization step, is a limiting factor to the description. One simplification of the analytical tasting task is to give the possibility to tasters to skip the verbalization step. That is why, for the app, two picture books have been designed. The aroma picture book (150 aromas) has been validated by a panel of 250 wine professionals. One picture has been validated for each major off-flavor of wine and for 150 flavors. The validation has been performed considering the recognition of the image, the proximity with the odor perceived in wine and the hedonic valence. Another picture book has been designed in order to help the taster to describe the global sensations perceived in month during three periods: the "attack"; the "middle" and the "final". The "dynamic in mouth" picture book has been validated by a committee of 12 wine professional tasters. It permits to the taster to generate a curve supposed to traduce the dynamic of wine in mouth.

The conception of the application and of the various visual interfaces, for us, a pretext to impose a new reflection on the tasting practices by the wine workers. Winemakers, traders, consultants, journalists were questioned about their tasting practices. A dozen of them were consulted on their tasting methods but also the quality criteria taken into account as a priority. Thus we feed our research on the quality of wines but also we revisit our approaches to the teaching of tasting. The observation of the terroir and the actors leads to understand the threats and forces, to make assumptions, to experiment, to conclude and adapt these conclusions to the needs of the field. Beyond a simple "app", ProGusto is a part of this reflection.

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## VII.P.11

# SENSORY CHARACTERISATION OF RED WINE IN NINGXIA REGION OF CHINA

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**Keywords:** Sensory analysis , Napping , Cabernet Sauvignon , Ningxia

With the increasing consumption of wine in China, the local wine industry is gradually developing. After making wine for thirty years, Ningxia region has been the most popular and promising wine region in China. Although a large quantity of wine has been produced, the style of Ningxia red wine has not yet been characterized. In the present study, wine samples of Cabernet Sauvignon and Merlot were collected from fifteen wineries located in four sub-regions of Ningxia region.

The vintages were ranged from 2013 to 2017. The sensory method of Napping was applied to describe the sensory characters of Ningxia red wine by the trained tasting panel. Appearance, aroma and taste evaluations were preformed, respectively. Several California and Bordeaux red wines were also analysed as control. The results showed that an obvious sensory characteristics of Ningxia red wine could be observed. A dried sweet potato aroma and vegetal aroma were found in Ningxia red wine. The descriptors of "juicy" and "less astringent" were often used in Ningxia red wine in the mouthfeel. Further study focusing on their chemical corresponding reasons is still under investigation.

## PREDICTING THE AGEING POTENTIAL OF CHAMPAGNE RESERVE WINES, DEVELOPMENT OF A NEW TOOL FOR SENSORIAL ANALYSIS.

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**Keywords:** Ageing potential, Champagne, Sensorial analysis, Prediction

The aging potential concept is used by experts and professionals tasting to describe a positively ability during aging of great red or white wines.<sup>1-4</sup> An examination of various psychological studies carried out on this subject reveals that this concept is organized with two important dimensions, the time and the quality. To describe the complex concepts the quantitative evaluation strategy, seems to be inappropriate.

From the definition of aged Champagne reserve wines formulated by Champagne wine-makers, a new method sensory analysis has been developed. This method include with two dimensions evaluation based on "ages" and "quality" scales. With the mandatory practice decree of wine aging by the AOC "Champagne" appellation the professional tasters from this geographical area had valuable experience to develop this method. Fast and instinctive for initiated and trained panel, the two dimensions allowed introducing several dependent variables, inseparable, for wine aging potential evaluation. Panel consensus measuring, wines discriminating, identifying several qualitative evolution types and judging the aging potential according to the current sensory wine age, were major strength and new parameters simultaneously integrated in a sensory analysis method. A dedicated statistical analysis has been developed to enable wines and judges scientific evaluations. Validated by a practical use during two vintages, this new tool allowed to precisely sorting the wines for their aging aptitudes. Useful in winemaking but also in scientific research, this transposable method has many other sensory concepts and opens new perspectives for the quantitative evaluation of complex concept in oenology.

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