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5th MS-Wine Day

May 22-24, 2024

Research Centre for Enology and Viticolture, Asti

TRACES IN THE GLASS
MASS SPECTROMETRY
FOR QUALITY AND PROFILING
IN WINES, FERMENTED BEVERAGES, AND DISTILLATES

Main topics

Wine and Fermented Beverages quality

Traceability and Counterfeit in Fermented Beverages

Metabolomic and Proteomic Profiles in Wine, Beer, and Spirits

Cutting-Edge MS Techniques Applied to Enology

Monitoring Oenological Processes

Contaminants and Faults in Alcoholic Beverages

5th MS-Wine Day

TRACES IN THE GLASS

MASS SPECTROMETRY FOR QUALITY AND PROFILING IN WINES, FERMENTED BEVERAGES, AND DISTILLATES

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May 22-24, 2024

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Asti 22-24 May 2024

Wednesday 22 May

13.30-14.30 Registration
14.30-14.45 Welcome

Topic – Volatomics and aroma compounds Section 1
Chairs M. Petrozziello – A. Asproudi

14.45-15.30 PL1 **Solving the Puzzle of Wine Aroma: An Analytical Chemistry Perspective**
Ricardo Lopez Universidad de Zaragoza, Spain

15.30-15.50 OR1 **Mass Spectrometry coupled with Gas Chromatography; a useful tool to characterize and typify the aroma characters of Greek variety Xinomavro**
Kotseridis Yorgos Agricultural University of Athens

15.50-16.10 OR2 **Volatomics-based classification of wines from different grape varieties, soil types and technological treatments using Partial Least Squares - Discriminant Analysis (PLS-DA)**
Matteo Pollon Università degli Studi di Palermo

16.10-16.40 **Coffee break**

Topic – Volatomics and aroma compounds Section 2
Chairs R. Flamini - F. Bonello

16.40-17.00 OR3 **Study on the release of 3-mercaptohexan-1-ol and the production of 3-mercaptohexyl acetate by yeasts in Asti wine**
Guido Bezzo Consorzio per la Tutela dell'Asti

17.00-17.20 OR4 **Application of yeast-based bio stimulants in vine: GC-MS determination of the volatilome in Vermentino and Sangiovese grapes and wines**
Andriani Asproudi CREA-VE Asti

17.20-17.40 OR5 **The volatile composition of wine is affected by the Hanseniaspora vineae-to-Saccharomyces cerevisiae inoculum ratio**
Mauro Paolini Fondazione Edmund Mach

17.45 Transfer to the Marchesi Alfieri Estate

18.30-20.30 **Welcome cocktail at the Marchesi Alfieri Estate**

Thursday 23 May - Morning

Chairs F. Magni – A. Panighel

9.00-9.45 PL2 **Technological advancements in forensic science: fostering interinstitutional Cooperation**
Antonietta Lombardozzi Polizia di Stato

Topic – No-wine products

9.45-10.05 OR6 **High-throughput targeted and untargeted headspace analysis of spirits by PTR-ToF-MS**
Iuliia Khomenko Fondazione Edmund Mach

10.05-10.25 OR7 **SPME-GC-MS screening of aromatic profiles in beers fermented with non-saccharomyces oenological yeasts**
Vasiliki Ragkousi CREA-VE Asti

10.25-10.45 OR8 **GC-MS Characterization of aromatic profiles of traditional Reggio Emilia balsamic Vinegar**
Federica Bonello CREA-VE Asti

Pillole di strumentazione

10.45 -10.55 **Beyond taste: Shimadzu's foodomics approach for wine profiling and classification**
Shimadzu Italia – Domingo Pastran

10.55-11.25 **Coffee break**

Topic – Enological practices and products

Chairs D. Caruso – M. De Rosso

11.25-11.45 OR9 **Effect of cold liquid stabulation on the volatile and sensorial composition of white wines**
Camilla De Paolis University of Turin

11.45-12.05 OR10 **Apparent matrix effect of commercial mannoprotein on the wine fruity esters during wine aging**
Calilin Kong DAFNAE, Agripolis, Università di Padova

Short presentations

12.05-12.10 SP1 **Volatile profile and antioxidant properties of Ruché wine from Piedmont**
Roberto Rabezzana Università di Torino

12.10-12.15 SP2 **Effect of storage time and temperature on the volatile composition of Malvasia di Candia Aromatica white wine by GC×GC-MS**
Leonardo D'Intino Università Cattolica del Sacro Cuore

12.15-12.20 SP3 **Base wines for sparkling wine from resistant varieties: effect of the altitude on the aromatic profile**
Mauro Paolini Fondazione Edmund Mach

12-20-12.25 SP4 **Aroma characterization of commercial Prosecco sparkling wines**
Davide Slaghenaufi Dipartimento di Biotecnologie Università di Verona

Pillole di strumentazione

12.25-12.35 **Profili aromatici: soluzioni analitiche avanzate per la caratterizzazione esaustiva quali/quantitativa e sensoriale**
SRA Instruments – Andrea Carretta Senior Food Application Specialist

12.40-14.00 **Buffet lunch**

Thursday 23 May - Afternoon

Topic – Wine Quality Section 1
Chairs M. Petrozziello – Susanna Rio Segade

14.00-14.20 OR11 **Evidence of the correlation between the Oxygen Radical Absorbance Capacity (ORAC) and the potential development of atypical aging (ATA) in white wines**
Roberto Larcher Fondazione Edmund Mach

14.20-14.40 OR12 **Climate Change effects on the occurrence of ochratoxin A in red and white commercial wines from the Greek territory**
Dimitrios Evangelos Miliordos Hellenic Agricultural Organization Demeter

14.40-15.00 OR13 **Exploring polysulfides in wine: formation, degradation, and implications for quality**
Tiziana Nardin Fondazione Edmund Mach

15.00-15.20 OR14 **The wine is "naked": Flint glass bottles cause wine aroma identity degradation**
Silvia Carlin Fondazione Edmund Mach

15.20-15.40 OR15 **Selective isolation of chemicals by a customized GC×GX setup**
Fabio Di Francesco University of Pisa

15.40-16.10 **Coffee break**

Topic – Wine Quality Section 2
Chairs A. Asproudi – R. Flamini

16.10-16.30 OR16 **Development of a metrological method for the absolute quantification of animal fining agents in Nebbiolo-based aged red wine by means HPLC-HRMS Q-Exactive Orbitrap**
Beatrice Aiuto CNR-ISPA

16.30-16.50 OR17 **Why PFAS in wine?**
Emanuele Ceccon Restek S.r.l.

Short presentations

16.50-16.55 SP5 **New approaches to traceability of oenological tannin origin: high-resolution mass suspect screening study**
Tiziana Nardin Fondazione Edmund Mach

16.55-17.00 SP6 **Evaluation of oenological strategies for the valorisation of methyl salicylate in Lugana wines**
Rosario Pascale Università di Verona

17.00-17.05 SP7 **Pathways to cineole formation in aged Amarone wines**
Jessica A. Samaniego Solis University of Verona

Pillole di strumentazione

16.05-16.15 **Fast analysis of haloanisoles in cork macerates and wines**
Bruker Italia S.r.l. Daltonics Division - Giuseppe Federico Labella

17.00 **Castle Visit**

20.00 **Social Dinner**

Friday 24 May

Topic – Characterization and authentication Section 1
Chairs R. Larcher – D. Caruso

9.00-9.45 PL3 **Advancements in Wine Metabolomics Research**
Panagiotis Arapitsas University of West Attica, Grece

9.45-10.05 OR18 **The state-of-the-art of authentication and traceability studies on Piedmontese red and white wines: the role of lanthanides**
Federica Gulino Università del Piemonte Orientale "A. Avogadro"

10.05-10.25 OR19 **MS as tool for selection of a fungus-resistant Glera grape crossing (downy mildew and powdery mildew) suitable to produce Prosecco wines**
Annarita Panighel CREA-VE Conegliano

10.25-10.45 OR20 **Sequential harvest of Vitis vinifera L. Mouhtaro grapes: influence in aromatic and sensorial characteristics**
Dimitrios Evangelos Miliordos Hellenic Agricultural Organization Demeter

10.45-11.05 OR21 **Study of aromatic and nutraceutical potential of six PIWI resistant vine varieties by high-resolution MS metabolomics**
Mirko De Rosso CREA-VE Conegliano

11.05-11.35 **Coffee break**

Topic – Characterization and authentication Section 2
Chairs F. Romaniello – T. Nardin

11.35-11.55 OR22 **LC-HRMS and 1H-NMR metabolomics data fusion as a tool for the comprehensive characterization of wine: A case study of Amarone**
Pier Paolo Becchi Università Cattolica del Sacro Cuore

11.55-12.15 OR23 **Variations in sensorially-relevant metabolites and indices in PDO wines of common genetic background: a case study on commercial Lambrusco wines**
Giovanni Luzzini University of Verona

12.15-12-35 OR24 **Study of anthocyanin evolution of a PIWI red wine undergone to oxidative conditions**
Riccardo Flamini CREA-VE Conegliano

Pillole di strumentazione
12.35-12.45 **Workflow automatizzati applicati ai sistemi LCMS di Agilent - Utilizzo di logiche di controllo reattive al servizio del laboratorio**
Agilent Technologies Italy - Lorenzo Zingaro LCMS Product Specialist

12.45-13.00 **Final remarks**

Oral Presentations
&
Plenary Lectures

PL1

Solving the Puzzle of Wine Aroma: An Analytical Chemistry Perspective

Ricardo Lopez

Laboratory for Aroma Analysis and Enology. Faculty of Sciences, Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza (Spain)

Keywords: *wine aroma, aroma analysis*

Aroma is one of the most important attributes of wine; a great wine can induce a perception of flavor complexity and intensity that is well-balanced and harmonious [1]. For this reason, wine aroma has been a topic of interest for scientists since the beginning of the 19th century [2]. However, wine is a complicated mixture of thousands of volatile compounds, of which aroma-relevant volatiles only constitute a minor subset of around one hundred. Therefore, for the complex task of wine aroma modelling, researchers need to determine all the relevant aroma compounds, whether acting alone or in concert, that are responsible for the wide range of aromatic sensory perceptions. This is the first unavoidable step, which should be complemented with information related with headspace concentration evolution and physicochemical interactions involving wine matrix components.

This communication seeks to define the challenges posed by wine aroma from the perspective of an analytical chemistry problem. Our goals are to define which compounds are the targets for quantification, at what concentrations levels they should be quantified, what chemical or physicochemical properties they possess that are useful from an analytical point of view, and finally, and considering all the previous questions, what solutions have been given or could potentially be given to solve this analytical problem.

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1. Ferreira, V., B. De La Fuente, and M.P. Saenz-Navajas, Wine aroma vectors and sensory attributes, in Managing Wine Quality. Volume I: Viticulture and Wine Quality. **2022**.
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OR1

Mass Spectrometry coupled with Gas Chromatography; a useful tool to characterize and typify the aroma characters of Greek variety Xinomavro

Goulioti Elli, Lola Despina, Kotseridis Yorgos

Laboratory of Enology and Alcoholic Drinks, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Greece

Keywords: *GC-MS, GC-MS/O, sensory analysis*

The quality of wines has often been associated with their geographical area of production. The relationship between wine character and its volatile composition is recognized by several researchers worldwide. Since these compounds influence consumers' sensory perceptions, volatile composition and sensory properties are essential in determining wine aroma characteristics. The aim of this work was to characterize the volatile composition and its corresponding aroma profile of the Amyndeon Protected Designation of Origin (PDO) Xinomavro red wines and then compare these data with the typical PDO Naoussa wines.

At first, volatile compounds of Amyndeon wine samples were isolated by liquid-liquid extraction, concentrated with Solvent Assisted Flavour Evaporator apparatus and analysed by gas chromatography-mass spectrometry (GC-MS) combined to olfactometry, in order to identify the key odorants of the variety. Then, to compare the wines came from the two PDO regions. a total of 25 aroma compounds were selected and quantified using gas chromatography-mass spectrometry (GC-MS) with simultaneous full scan and selected ion monitoring for data recording, and then odor activity values (OAVs) were determined. Also, a trained panel evaluated the wines using sensory descriptive analysis, rating a total of 13 aroma attributes.

According to the Olfactory analysis, 30 aroma-active compounds were identified, of which, ethyl hexanoate had the highest modified detection frequency (MF%). The quantitative data proved that Xinomavro wines have a complex aroma profile rich in higher alcohols, ethyl esters, acetate esters and fatty acids, with a contribution of terpenes and volatile phenols. Statistical data analysis techniques showed the structure of the experimental data and the significant differences for each compound in the regional wines. PDO Amynteon wines presented higher concentrations in 1-hexanol and higher intensity of green bell pepper attribute, while PDO Naoussa wines were higher in ethyl octanoate, ethyl 2-methylbutyrate and eugenol, with higher scores in berry fruit and spices attributes.

This study provides an approach to the chemo-sensory fingerprinting of Xinomavro PDO wines. It will be further used to advance the understanding of how impact aroma compounds and sensory characteristics shape terroir expression. Actually, a new GC-MS/MS method is developed and applied for the quantitative analysis of even more volatile compounds, aiming to gain insights on the aroma of Xinomavro wines by analysing compounds at lower concentrations too.

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OR2

Volatomics-based classification of wines from different grape varieties, soil types and technological treatments using Partial Least Squares - Discriminant Analysis (PLS-DA)

Matteo Pollon, Clara Vitaggio, Manuel Schnitter, Valentina Caraci, Luciano Cinquanta, Onofrio Corona

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Viale delle Scienze 4, 90128, Palermo, Italy

Keywords: *volatile compounds, sur lies, PLS-DA*

Enhancing the value of wine products also involves identifying both abiotic and biotic factors that shape their features. By formalizing how these factors influence wine composition, it can be possible to characterize wines from specific regions, communicate these unique traits to consumers, develop tailored winemaking approaches to preserve regional features and develop models to identify some frauds (Aceto et al., 2018; Feher et al., 2019).

To study how some important biotic and abiotic factors influence the wine composition, we designed a factorial experimental plan encompassing four main factors: grape variety, soil type, oenological treatment and aging duration. We conducted analysis of the free volatile components using solid-phase extraction on silica with a C18 polymer, followed by GC-SQMS analysis (Corona, 2010). The grape varieties studied were *Grillo* and *Chardonnay*, while the soils examined were categorized as limestone and marlstone. Oenological treatments included maturation on fresh lies, on mature lies and control without any lies added. While the aging time was one, four, and seven months.

For data analysis Partial Least Squares – Discriminant Analysis (PLS-DA) was employed. To streamline the model for practical application and interpretation, a pre-selection of independent variables was implemented, excluding those with a Variable Importance in Projection value (VIP) below one on the first component in the initial PLS-DA fit and re-fitting a second reduced model.

Regarding grape variety, we achieved 100 % of correct classification using 16 variables. For soil type, our model, based on 11 variables, correctly classified 91.67% of samples. Concerning sur lies treatment, an initial analysis with 23 variables revealed no distinction among samples after one month of treatment nor between those treated with fresh or mature lies at four and seven months. Consequently, we excluded one-month-old samples and combined fresh and mature lies treatments, resulting in a PLS-DA model with 100% predictive accuracy.

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OR3

Study on the release of 3-mercaptohexan-1-ol and the production of 3-mercaptohexyl acetate by yeasts in Asti wine

Guido Bezzo [1]***, Mariachiara Lo Scalzo*** [2]

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Keywords: *Moscato bianco, 3-mercaptohexanol, 3-mercaptohexyl acetate*

The presence of odorous thiol compounds in wines derived from the white Moscato grape grown in the Asti DOCG area has already been previously reported at an organoleptic level, by research carried out on the production of "Asti in reduction" [1]. In the present study, the presence of both the two precursors cysteinyl and glutathionyl derivatives of the thiol 3-mercaptohexanol (3MH) and the free and odorous form of 3MH and 3-mercaptohexyl acetate (3MHA), present in the derived wines from the white Moscato grape, was analytically confirmed. Furthermore, the effect of the yeasts *S. bayanus* and *S. cerevisae* on the release of 3MH and its transformation into 3MHA during fermentation and after 4 weeks of aging was studied. The instrumental analysis was carried out in HPLC-MS/MS, on the diluted sample for the analysis of the precursors and instead after derivatization with ethyl propiolate (ETP) for the free forms, in order to obtain a better separation of the compounds and an increase selectivity and sensitivity in complex matrices such as must and wine. The data were acquired in MRM allowing for good sensitivity for quantification. The quantitative determination of the precursors was conducted using as a reference the data obtained from the analysis carried out on the must before micro-vinification. The free compounds 3MH and 3MHA have been found at concentrations above their olfactory perception threshold, with aromas reminiscent of passion fruit and grapefruit [2]. This evidence was also confirmed by the organoleptic analysis performed simultaneously with the instrumental analysis. During fermentation the best results were obtained, both for 3MH and 3MHA, in the thesis inoculated with S. *bayanus*. During the first 4 weeks of refinement, 3MH was released more in the wine produced with *S.cerevisiae* without the addition of Cytolase enzyme, reaching a maximum in the fourth week. The acetylation of 3MH with generation of 3MHA showed its maximum in the sample treated with *S. bayanus* without addition of Cytolase as expected based on the metabolic activities of this yeast. The sample inoculated with *S. cerevisiae* without the addition of Cytolase also showed continuous growth, although less than the other yeast.
It can therefore be concluded that during the first month of refinement it is possible to detect an increase in odorous thiols with the rest of the wine on the lees. The presence of these highly odorous molecules expands the possibility of producing wines whit new characteristics. This study also reveals previously unknown compositional information about a product of international importance.

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OR4

Application of yeast-based bio stimulants in vine: GC-MS determination of the volatilome in Vermentino and Sangiovese grapes and wines

Andriani Asproudi[1], Maurizio Petrozziello[1], Loretta Maria Panero[1], Vasiliki Ragkousi[1], Mauro Eugenio Maria D'Arcangelo[2], Federica Bonello[1]

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Keywords: *aroma precursors, Vermentino, Sangiovese*

In a context of climate change and overuse of agrochemicals, sustainable approaches such as the use of bio stimulants offer a viable alternative for viticulture, helping withstand abiotic and biotic threats in viticulture. Bio stimulants are organic compounds, microbes, or a combination of both, that generally enhance plant processes enabling high yields and high-quality products. In vine, may stimulate a natural immune response that produces secondary metabolites key compounds for the organoleptic quality of grapes and wines.
During this research the prospect of foliar application of yeast-based bio stimulants to improve the aromatic quality of Vermentino and Sangiovese grapes and respective wines was investigated. The experiment involved two cultivars cultivated in Tuscany, a white (Vermentino) and a red one (Sangiovese). Two different products, prepared with specific fractions of inactivated yeasts, were compared, and applied in different points during veraison with two or three-time application protocol. The quali-quantitative determination of the aromatic composition of grapes and respective wines was conducted using Solid Phase Extraction (SPE) for sample preparation, followed by Gas Chromatography-Mass Spectrometry (GC-MS).
The application of biostimulants did not influence the overall yield of the vines; however, an increase in berry weight and a reduction in sugar content were observed in grapes harvested from treated vines. In the first year of study, bio stimulants application, triggered significantly higher contents for almost all chemical classes of aromatic precursors for Vermentino grapes and enhanced the accumulation of terpenoids and benzenoids in the berries of Sangiovese. Vermentino wines from treated grapes appeared to be characterized by a significantly higher content of ethyl and acetic esters, as well as terpenes and norisoprenoids, compared to the control wine. As regards the Sangiovese wines, fermentation compounds like ethyl esters and acetic esters, particularly isoamyl acetate, were found to be significantly higher in those from treated thesis. In the second year of study, the effect of biostimulants was not as pronounced as in the first year. Some significant differences in favor of the treated grapes were confirmed regarding terpenoids in both grape varieties. In wines also, no marked differences were noticed except for the significantly higher contents of evolution esters for the wines from treated vines.
Bio stimulants application, thus, seems to improve the qualitative parameters of the grapes and wines, but their effect was different based on the type of bio stimulant, vintage year, chemical class of compounds and cultivar examined. The way vines absorb and metabolize biostimulants could change over time, potentially due to modifications in vine health or soil conditions and this may result in less pronounced effects in subsequent years. Further future investigation is necessary to optimize bio stimulant application to contrast stress conditions and improve grape quality.

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OR5

The volatile composition of wine is affected by the *Hanseniaspora vineae*-to-*Saccharomyces cerevisiae* inoculum ratio

Mauro Paolini [1], Adelaide Gallo [1,2], Nicola Cappello [1], Roberto Larcher [1], Tomas Roman [1]

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Keywords: *mixed fermentation, β-phenylethyl acetate, non-Saccharomyces*

The non-*Saccharomyces* yeast, *Hanseniaspora vineae* Hv205 (Hv), is an apiculate yeast proposed for winemaking due to its good alcohol tolerance. Moreover, its metabolic characteristics can enhance some organoleptic traits, contributing to the aroma and texture of wines. Previous studies have demonstrated that sequential inoculation of Hv and *Saccharomyces cerevisiae* (Sc) leads to the overproduction of ß-phenylethyl acetate, associated with an increase in rose-like hints in wine. However, sequential inoculation management with Sc could be complicated in white winemaking [1]. In this study, the fermentation of a Glera must was performed with an initial inoculum of 2×10^6 CFU/mL at five different co-inoculation ratios of Hv and Sc: 67%, 80%, 90%, 95%, and 98%. The kinetics of fermentation and wine aroma were compared with the results obtained from pure inoculation with Sc.
Compared with Sc, the presence of Hv accelerated fermentation until 30% of the process was reached, regardless of the co-inoculation ratio. After that point, the superior performance of Sc became most apparent, leading to an earlier completion of fermentation. Moreover, increasing the Hv ratio slowed sugar consumption rate, and the 67% Hv protocol delayed only 2% the time needed to complete fermentation in comparison with the pure inoculum of Sc.
The GC-MS/MS volatilome analysis [2] confirmed that both the presence of Hv and the co-inoculation ratio significantly affected the content of specific yeast-derived aroma compounds in wines. Regarding 2-phenylethanol, Sc wines showed the highest values (~20 mg/l), followed by the 67% Hv protocol (~11 mg/l), and were lower in the rest of the trials (~8 mg/l). Regarding its acetate, the results were reversed: increasing Hv ratio led to a higher content of ß-phenylethyl acetate in wine, racing 20-folds the concentration obtained with only Sc. Concerning isoamyl acetate, the pure Sc fermentation was comparable to those with a higher Hv inoculum (90%, 95%, 98%), while the 67% and 80% inoculations were even higher. The concentration of ethyl hexanoate was affected by yeast species, and Sc produced up to 3-folds the concentration compared with the Hv protocols, for which it was not found any difference between them.
Interestingly, the concentration of the four yeast-derived metabolic markers was linearly correlated with the co-inoculation ratio: positive for ß-phenylethyl acetate and negative for the other markers. Overall, Hv co-inoculum can be considered a good alternative to sequential inoculation, shortening fermentation time while maintaining the Hv aroma characteristics.

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PL2

Technological advancements in forensic science: fostering interinstitutional Cooperation

Antonietta Lombardozzi

Polizia di Stato

Effective forensic investigations rely on seamless collaboration between diverse institutions, notably law enforcement agencies and scientific bodies. This abstract explores the significance and outcomes of interinstitutional cooperation in advancing forensic methodologies, emphasizing partnerships between forensic scientists and scientific police units. Such alliances facilitate the exchange of knowledge, resources, and specialized expertise, resulting in heightened investigative capabilities and precise forensic analyses. By utilizing the advanced techniques of scientific policing alongside the scientific rigor of forensic labs, law enforcement agencies can adeptly address intricate criminal cases, including homicides. Case studies and best practices in collaborative efforts underscore the crucial role of communication, mutual respect, and standardized protocols in fostering productive partnerships. Challenges such as resource limitations and maintaining impartiality are discussed, highlighting the necessity for ongoing collaboration and innovation. Notably, the integration of techniques like mass spectrometry emerges as a key factor in strengthening forensic analyses and enhancing criminal justice outcomes. In essence, cohesive interinstitutional collaboration remains essential for the pursuit of justice and truth in contemporary forensic investigations.

OR6

High-throughput targeted and untargeted headspace analysis of spirits by PTR-ToF-MS

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Keywords: *direct injection, volatile organic compounds, alcoholic beverages*

Spirits are the type of alcoholic beverages produced by distillation and, therefore, contain a higher amount of ethanol. Consumers widely appreciate characteristic liquor aroma and flavour. Its composition, formation, and origin are the subject of specific studies in food science. Moreover, standards and regulations related to spirit drinks have been established by different countries and international organizations to ensure their safety and quality, introducing the maximum levels of some naturally occurring or added compounds, such as methanol.
The gold standard method for the determination of volatile organic compounds (VOCs) in alcoholic beverages is gas chromatography with its drawbacks in terms of labour and time consumption. PTR-ToF-MS, being a direct injection technique, is a powerful tool for measuring VOCs emitted from the different food matrices. However, the high ethanol content present in alcoholic beverages is an issue for PTR-MS. Romano *et al*. collected possible strategies to overcome this problem [1].
In this study we tested the use of Argon as buffer gas as one of the proposed approaches [1] in a SHS module coupled to a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) and PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) to evaluate VOCs content of 32 different liquors, such as grappa, tequila, mezcal, gin, brandy and alcohol. Additionally, the methanol quantification was performed in three different hydro-alcoholic solutions. 5 µL of each sample were transferred into a 20 mL vial in triplicate.
The method employed in this study allowed to measure the alcoholic beverages from 39 to 96% of ethanol without changing of PTR-MS settings. A PLS-DA model was developed to distinguish different types of liquors. According to the model VIPs, the tentatively identified methyl furfural (*m/z* 111.047 – $C_6H_6O_2H^+$) and dimethylnaphthalene (*m/z* 157.107 – $C_{12}H_{12}H^+$) were involved in the separation of spirits made from the agave plant (mezcal and tequila). Various monoterpenes were present in all gin and some grappa samples. Moreover, a PLS-R model for methanol content was built, and the highest VIPs were *m/z* 33.032 (t.i. methanol, VIP score = 12.018) and *m/z* 48.052 (t.i. isopologue of ethanol, VIP score = 10.551).
Despite the spirits' high ethanol concentrations limiting the PTR-MS performance, it is still possible to obtain valuable insights. High-throughput targeted and untargeted headspace analysis of spirits by PTR-ToF-MS can be relevant for quality control or monitoring fermentation and distillation processes. This study highlights the effective utilization of PTR-MS even in suboptimal conditions characterized by high ethanol content.
The present work has been developed in the framework of the projects 'ONFOODS', [Italian National Recovery and Resilience Plan (NRRP) projects financed by the European Commission's Next Generation EU programme].

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OR7

SPME-GC-MS Screening of Aromatic Profiles in Beers Fermented with Non-Saccharomyces Oenological Yeasts

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Keywords: *SPME-GC-MS, aromatic profiles, oenological yeasts*

The brewing industry is increasingly embracing diversity with a growing interest in utilizing novel starter cultures. These microorganisms, sourced from various indigenous biological resources, are capable of imparting unique and distinguishable sensory characteristics to beer. Current research focuses on exploring microbial biodiversity for beer production [1] with the aim to identify novel microorganisms that could enhance the quality and distinctiveness of the final products. Notably, non-*Saccharomyce*s yeasts have shown potential in imparting unique organoleptic properties to beer and are also being explored for low-alcohol beer production, a rapidly expanding market segment. This study integrates various brewing processes, technologies, and ingredients that have been developed globally over recent decades [2], contributing significantly to the field.

In this work, the contribution of 31 yeast strains from the Collection of Microorganisms of Viticultural and Oenological Environment (CREA-CMVE), along with two commercial non-Saccharomyces strains, to the volatile profile of malt-based fermentations was assessed using Solid Phase Microextraction coupled with Gas Chromatography-Mass Spectrometry (SPME/GC-MS). Strains from *the Zygosaccharomyces, Kazachstania*, and *Metschnikowia* genera exhibited a notable propensity to produce higher alcohols, such as isoamyl alcohol and phenylethyl alcohol, influencing the beer's aromatic profile significantly. It must be noted, however, that high concentrations of these compounds may lead to an overly robust olfactory impact. Alternatively, species such as *Lindnera saturnus*, *Pichia kluyveri*, and *Meyerozyma caribbica* were distinguished by their pronounced synthesis esters, like isoamyl acetate and 2-phenylethyl acetate. This capability could enhance the beer's aroma with floral and fruity notes, though it may also produce solvent-like notes if very high concentrations are achieved. Furthermore, an intricate relationship between yeast strains and hop-derived isoprenoids, including terpenes and sesquiterpenes, was observed. *Metschnikowia* and *Candida* species were implicated in the enhancement of these compounds in the beverage. The correlation between linalool accumulation and fermentations by *Starmerella* and *Hanseniaspora* is especially promising for aroma modulation.

This investigation underscores the pivotal role of specific yeast species in flavor and aroma development during beer fermentation and suggests new strategies for crafting distinctive beer profiles through yeast selection.

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OR8

GC-MS Characterization of Aromatic Profiles of Traditional Reggio Emilia Balsamic Vinegar

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Keywords: *vinegar, aromatic compound, wood*

This research was aimed to characterize the peculiar aromatic profile of the traditional balsamic vinegars from Reggio Emilia (ABTRE, Aceto Balsamico Tradizionale di Reggio Emilia). The production ABTRE follows a meticulous process that begins with the cooking of the grape must and continues through a series of fermentations and aging stages in barrels made of different woods that, contributes to the development of unique aromatic and flavor profiles in the vinegar as it matures over time. This process a result of a centuries-old Italian enogastronomic tradition, highlights the unique properties of traditional balsamic vinegar compared to other types of vinegar.

Traditionally, ABTRE is refined in a battery of barrels consisting of six containers constructed from various woods and arranged in decreasing volume. The woods utilized for these barrels are mulberry, cherry, chestnut, ash, black locust, and oak. Various batches of vinegar, initiated between 1991 and 2009, were systematically examined. The samples were analysed following extraction via solid-phase extraction (SPE) and using advanced techniques such as gas chromatography and mass spectrometry [1,2]

During aging, the aromatic compounds derived from alcoholic fermentation, such as ethyl esters, decrease dramatically, while those associated with acetic biosynthesis, oxidative aging and the Maillard reaction increase.

A distinctive element of traditional balsamic vinegars is the predominant presence of aldehydes and acetals. Namely vanillin and phenylacetaldehyde are responsible for unique olfactory notes such as, vanilla, honey and spice aromas. Other significant compounds include 5-hydroxymethyl-2-furfural, suggested as a marker for vinegar authenticity, reaching relevant concentrations only after six years of aging.

In summary, the outcomes of these studies offer a more comprehensive insight into the complex chemical processes that transpire during the production of balsamic vinegar. They also underscore the critical role of scientific methodologies in enhancing and certifying traditional Italian products.

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OR9

Effect of cold liquid stabulation on the volatile and sensorial composition of white wines

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Keywords*: pre-fermentative technique, VOCs, GC-MS*

Wine is a complex beverage characterized by different chemical compounds and components that together can have an impact on product final quality. Different winemaking technologies are used to modify basic parameters, phenolic and aromatic profile to fully exploit the grape qualitative potential. Particularly, in white wine production, pre-fermentative processes are applied to increase the extraction and to preserve grape's native compounds. Cold liquid stabulation (CLS, *macération sur bourbes*) is an innovative technique that consists of maintaining grape juice on its lees, in suspended conditions, for a variable time at low temperature [1]. In this study, the CLS was applied on two non-aromatic white grape varieties: 'Arneis' and 'Cortese'. The aim was to evaluate the CLS effect on phenolic, color, antioxidant, and aroma features of produced wines. 'Arneis' and 'Cortese' grapes, after destemming and crushing, were pressed and the juices kept on their lees, manually stirred twice a day, at 4 °C. Three stabulation lengths (in three replicate each) were tested: 7, 14, and 21 days, and compared with a non-stabulated control. At the end of stabulation, the juices were racked, fermented, cold stabilized, and bottled. In particular, free and glycosylated volatile compounds (VOCs) were purified by solid phase extraction (SPE) and then determined using gas chromatography-mass spectrometry (GC-MS) [2]. Selected acetate and ethyl esters, higher alcohols, acids, sulphur compounds, C6 compounds, terpenes, norisoprenoids, benzenoids, and volatile phenols were studied. Basic (soluble solids, acidity, ethanol), color (CIELab), phenolic, and antioxidant (DPPH) parameters were also analyzed at the end of CLS, at the end of fermentation, and one month after bottling [3,4]. The chemical data were compared with sensory analysis performed on produced wines by a trained panel with a mixed approach of descriptive analysis (DA) and Check-all-that-apply (CATA) strategies. The results showed that the CLS had an impact on the acidic composition of the wines for both varieties, lowering the tartaric acid content already after 7 days of treatment. The CLS-treated wines did not differ in terms of color compared to control and showed limited differences in total polyphenols, without influencing the perceived astringency and bitterness. Different grape phenolic composition likely has a higher impact than the application of CLS treatment. Regarding VOCs, the results are contrasting depending on the CLS length and also on the variety. Total glycosylated VOCs decreased in all treated 'Arneis' must samples, while they increased in stabulated 'Cortese' musts after 14 and 21 days. In wines, no differences in total VOCs were found with the CLS length, although the concentration of free terpenes increased after bottling for 21 days of stabulation in 'Arneis'. Moreover, 'Arneis' esters were lower after 14 days of stabulation, particularly isoamyl acetate and 2-phenylethyl acetate, responsible of fruity and floral notes. In contrast, 14 and 21 days stabulated samples showed a higher content of isoamyl alcohol and 2-phenylethanol. Higher liking scores were reported for Cortese wines with 14 and 21 days of treatment. Probably, the lower content of free C6 compounds (-12%) and sulphur compounds, in particular 1-hexanol responsible for herbaceous hints and methionol, contributed positively on sensory properties. Together, the higher richness in individual ethyl esters such as ethyl octanoate, ethyl 4-hydroxybutanoate, and ethyl-3-hydroxybutanoate contributed to the fruity notes.

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OR10

Apparent matrix effect of commercial mannoprotein on the wine fruity esters during wine aging

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Keywords: *mannoprotein, wine fruity esters, wine aging*

The long-term storage of wine affects its volatile compound profile, leading to alterations in the overall aroma*1,2*. Fruity esters are volatile compounds that contribute to the aroma of wine and play an important role in maintaining aroma stability*3*. Building on evidence that spent yeast polysaccharides could impact the stability of wine esters*2*, this study aims to further investigate the role of mannoproteins (MPs) in maintaining the stability of these esters. Commercial MPs and MPs purified from the alcoholic fermentation of synthetic grape juice were used in the study. Their composition and molecular weight distribution were analyzed using UV spectrophotometry, electrophoresis, and high-performance liquid chromatography. The analysis revealed a total protein content of approximately 25% for the commercial MPs, with their molecular weights primarily distributed between 40-180 kDa and 7.5-40 kDa. To examine the influence of MPs on the stability of fruity esters, concentrations of 200 and 400 mg/L of MPs were added into a model wine system containing 12% ethanol, 5 g/L tartaric acid, and pH 3.0. This model wine was then spiked with various esters (ethyl isobutyrate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl phenylacetate) added at concentrations naturally found in wine. Through accelerated aging at 50°C for two and a half months, the concentration of these esters was determined by liquid-liquid extraction and gas chromatography-mass spectrometry. The findings show a hydrolysis rate of 92% for isoamyl acetate and between 64% and 82% for other ethyl esters after 1 month, with continued hydrolysis observed until the end of the period. The addition of MPs was found to slightly retard this hydrolysis, with the 400 mg/L concentration proving more effective than 200 mg/L. Further investigation in a real wine system, as well as the analysis of the sensory impact, is necessary to ascertain the role of MPs on wine aroma.

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OR11

Evidence of the correlation between the oxygen radical absorbance capacity (ORAC) and the potential development of atypical aging (ATA) in white wines

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Keywords: *ATA, ORAC, wine*

Atypical aging (ATA) is a recognized wine flaw characterized by unpleasant odors resembling wet rag, soap, wax, furniture varnish, and dishcloth [1]. These odors are primarily caused by the compound 2-aminoacetophenone (AAP), originating from the oxidative breakdown of 3-indoleacetic acid [2]. In this study, white wines of varying commercial grades and origins underwent evaluation for their Oxygen Radical Absorbance Capacity (ORAC) and susceptibility to ATA development during accelerated aging. It was hypothesized that winemaking practices might influence these parameters. Hence, the impact of oxygen exposure during fermentation and the post-fermentation addition of antioxidants was investigated on ORAC and ATA development. The potential correlation between ORAC and ATA was extensively analyzed across white wines of diverse qualities and origins.

ORAC assays were performed adapting previous methods that used fluorescein as fluorescent probe, while the IAA and AAP were determined using an Ultimate R3000 UHPLC coupled to a Q-ExactiveTM mass spectrometer (Thermo Scientific, Sunnyvale, CA, USA).

Fourteen musts were vinified in two distinct ways, and the ORAC and ATA development were evaluated to determine how exposure to oxygen during fermentation could affect the oxidative wine reaction. Additionally, the direct impact of ascorbic acid (Asc) addition to wine after vinification was investigated for its effect on ORAC and the subsequent protective effect against AAP formation during forced aging (6 days, 40°C). Oxygen protection during fermentation did not significantly affect the final ORAC or ATA development in wines. Conversely, the addition of ascorbic acid post-fermentation resulted in increased ORAC and reduced AAP formation.

Finally, the analysis of 120 Italian DOC white wines (comprising 6 varieties) and 45 table wines from various Italian regions revealed a tendency towards a negative correlation between wine ORAC and potential ATA development. This suggests that wines with higher ORAC values might be more shielded against AAP formation stemming from the oxidative degradation of IAA. Wines with ORAC values exceeding the experimental threshold of 3000 μM TE showed no signs of this potential defect, and none of the DOC wines, typically associated with meticulous winemaking practices, exhibited significant AAP levels during accelerated aging. In summary, ORAC could serve as a quick and cost-effective tool for predicting this potential risk in commercial wines.

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OR12

Climate Change effects on the occurrence of ochratoxin A in red and white commercial wines from the Greek territory

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Keywords: *Greek wine, ochratoxin A, climate change*

Wine plays a significant role in the economic development of several countries. Nevertheless, it is worth noting that wine can be subjected to contamination caused by mycotoxins generated by specific fungal species. Most available information on mycotoxins found in wine is coming from research conducted in countries from the Mediterranean basin [1]. It is important to highlight that grapes can easily be infected by mycotoxigenic fungi, particularly *Aspergillus carbonarius*, which is primarily responsible for the presence of ochratoxin A (OTA) [2]. In terms of contamination, it is crucial to recognize that climate serves as the most influential factor once these fungi have established themselves, with elevated temperatures serving as a significant contributor to OTA contamination. Wine samples of different type (white, rose and red), dry, from different regions of Greece (Northern Greece, Central Greece, Peloponnese, and Crete) were analyzed. Wine samples originating from Greek (Assyrtiko and Xinomavro) and international (Syrah and Sauvignon blanc) nobble grapevine varieties from 2020 and on, were analyzed using a HPLC analytical method developed to detect and quantify OTA using a modified QuEChERS extraction protocol [3]. Moreover, conventional parameters were measured according to OIV (2020) [4]. Interestingly, mycotoxin-contaminated wines showed low concentrations (<2 ppb). Higher concentrations of OTA were recorded in red wines in comparison to the white ones. In addition, the highest OTA concentrations were recorded in wines produced in areas either with high mean annual temperatures, Santorini island (19.1 °C) for white and Halkidiki (16 °C) for red wines or with high rainfall (Drama 842 mm) for white wines. Consequently, it is observed that concentrations of OTA in wine are relatively higher in the warmer regions of Greece as compared to the cooler areas. The effect of climate change on vines and mycotoxins in wine needs urgent consideration by well-constructed modelling studies and efficient interpretation of existing data. The evaluation of OTA presence in grape products originating from various cultivars and regions is imperative in order to ascertain the potential risk of human exposure to these chemical compounds.

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OR13

Exploring Polysulfides in Wine: Formation, Degradation, and Implications for Quality

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Keywords: *polysulfide, H_2S, Orbitrap*

In recent times, there has been a surge of interest in polysulfides (PSs) in wine, prompting several studies to investigate the possible factors and mechanisms behind this relatively unexplored aspect of oenology. One of their most interesting aspects concerns the formation of H_2S from the degradation of PSs, as it has the potential to cause defects or diminish the quality of the wine. While several glutathionylated and cysteinylated PSs have been identified in actual wine samples [1,2], studying these compounds has proven challenging due to various factors: they exist in low concentrations in wines, are chemically unstable, and pure standards of the individual compounds (RSnR with n > 2) are not commercially available.
In this study, the accumulation of cysteinylated and glutathionylated PSs during fermentation was monitored in synthetic grape media and Chardonnay juice using an ultra-high-pressure liquid chromatographer coupled with high-resolution mass spectrometer (UHPLC-HRMS) and an in-house database for suspect screening process.
Afterward, di-cysteinyl pentasulfide (CS_5C) was isolated using an integrated fraction collector, and its degradation was induced by exposure to 30°C and analyzed using UHPLC-HRMS. Concurrently, H_2S formation was measured using ion-exchange chromatography coupled with an amperometric detector (IC-PAD). The degradation of the various PSs was observed and, for the first time to the authors' knowledge, the release of H_2S originating from the CS5C target compound.
Finally, 57 sparkling wines from different vintages, including 2018, 2019 and 2020, were examined for their presence of glutathionyl and cysteinyl polysulfides with up to 5 sulfur atoms. The wines were evaluated after opening of the bottles at the same moment, directly before their analysis. The results revealed, for the first time, the evidence of the presence of polysulfides in natural, unspiked sparkling wines. Moreover, a potential effect of the ageing times and vintage was observed from the preliminary study on the differences between the different vintages.

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OR14

The wine is “naked”: Flint glass bottles cause wine aroma identity degradation

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Keywords: *GC×GC-Tof-MS, lightstrike*

Transparent packaging is often used for food products, including wine, milk, beer and fruit juices. This choice is based on the marketing recommendation that consumers want to see the product before they buy it, although scientists point out that light can harm food quality and nutritional value.
Although the practice of bottling white wine in clear glass is known to cause a wine defect, the influence of light on the fruity and floral flavor profile of the wine is unknown. The aim of this work was to study the influence of light exposure on the white wine volatilome under the typical supermarket shelf conditions and to monitor the primary aroma compounds that characterize the sensorial identity and flavor of each cultivar using 1,052 bottles of 24 white wines [1]. The volatile profile was studied using a fingerprinting method able to maximize the number of volatiles detected, via comprehensive gas chromatography combined with time-of-flight mass spectrometry (GC×GC-ToF-MS) instrument.
After only 7 days of shelf life in flint glass bottles, a dramatic loss of terpenes (10 to 30%) and norisoprenoids (30 to 70%) was recorded, while colored glass bottles did not show such behavior. even after 50 days and the darkness has preserved the fruity and floral aromatic integrity of the wine. Flint glass bottles bring no benefit to the wines, while the multiples change in the aroma composition can jeopardize the quality, depriving the wine of the identity of the variety and terroir. In other words, the wine is naked. In light of this understanding of the negative impact of flint glass on the aromatic identity and sensory character of white wine, this packaging should be strongly discouraged. The same results should apply to a wide range of different foods consumed daily in which clear packaging is used.

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OR15

Selective isolation of chemicals by a customized GC×GX setup

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Keywords: *GC×GC, 4-ethylphenol, 4-ethylguaiacol*

A novel approach capable of isolating and recollecting nearly any molecule from complex GC×GC chromatograms is presented [1]. This result was achieved by customizing a GC×GC-Q-TOF system with a Deans switch, a passive splitter, and careful adjustments of flows and capillary dimensions. The setup was evaluated with more than a hundred standard chemicals covering a wide range of volatility (boiling points: 56 – 343 °C) and polarity (log P: -0.2 – 9.4). We found that recovery from tube to tube can become highly efficient if a custom-made adapter is attached directly on the FID port (average recovery rate of 76 ± 7%). Furthermore, we could achieve an isolation down to a minimum distance of 50 ms between baseline separated eluting peaks. In addition, the setup was designed for easy adaptation by repurposing existing instrument control software to define the isolation windows for the compounds of interest (first and second column retention time windows). We expect this novel development to allow several new applications, e.g., the isolation or selective enrichment of molecules in food and flavour analysis; the investigation of suspect chemicals (incl. unknowns) for effect directed analysis (e.g., bioassays), and the isolation of chemicals for further chemical analysis. An illustrative example relevant to the removal of 4-ethylphenol and 4-ethylguaiacol from the headspace of a spoiled wine lot is shown.

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OR16

Development of a metrological method for the absolute quantification of animal fining agents in Nebbiolo-based aged red wine by means HPLC-HRMS Q-Exactive Orbitrap

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Keywords: *absolute quantification, trace amount, isotope dilution mass spectrometry*

Fining agents, such as egg albumen and gelatin, are added throughout the wine-making process to increase the wine stability over time and enhance the final product colour, flavour, and astringency. Nevertheless, the final clarified product may pose a risk to consumers who are highly sensitive to these proteinaceous adjuvants. This is because the application of proteinaceous fining agents not only causes the precipitation of tannins but also has the potential to promote the formation of soluble complexes between proteins and tannins. Several investigations have demonstrated that even in adequately processed clarified wine, traces of these complexes remain as residues [1;2]. To quantify traces of these fining agents and overcome allergological or food ethics problems, it is important to develop sensitive and precise detection methods. Immunochemical tests (ELISA), PCR analysis, and analytical determination based on mass spectrometry (MS) are the techniques most frequently employed to quantify allergen traces in food. These analytical assays, however, consider different analytes, such as protein epitopes, DNA fragments, and peptide markers. As a result, it becomes crucial to establish a common measurand to guarantee the result traceability and comparability which was defined by the provisions of Regulation (EC) no. 178/2002 (General food legislation) as milligrams of total allergen per kilogram of food [3]. The objective of our research is to provide a reference approach for the absolute quantification of fining agents in red wine using MS techniques.
Isotope dilution MS (IDMS) is a primary ratio method that relates to the SI with a direct small measurement uncertainty [3]. We started with the rigorous selection of the target peptides which is the first step for the analytical method. The proteins were reduced, alkylated, and digested with trypsin. An HPLC-HRMS DDA (Data Dependent Analysis) on albumen and gelatin standards was carried out, and 5 distinct and specific peptides (from 3 albumen and 2 gelatin proteins) were chosen. The peptides were selected for the absence of both missed tryptic cleavage sites and post-translational modifications. Moreover, they must be species-specific for each fining agents, as determined by BLAST searches. We decided to use a quantitative approach using an isotopically labelled form of the target analyte as internal standard [4]. The MS setting of the Parallel Reaction Monitoring (PRM) was performed by the individual flow injection analysis of the synthetized peptides by mass spectrometry and the selection of optimal collision energy (CE) values allowed to achieve maximum target transition sensitivity. Each synthetized peptide was detect-ed down to a level of 25 µg/L using the high resolution and mass accuracy of the PRM analysis. The workflow will proceed with the creation of a calibration curve using synthesized peptides (internal standard) and with the validation of this procedure. The Amino Acid Analysis (AAA) will assign an uncertainty value for each peptide to achieve metrological traceability to the SI.

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OR17

Why PFAS in Wine?

Emanuele Ceccon

Rested S.r.l.

Keywords*: wine, pfas, vegetables*

The presentation will cover the request coming from Italian govern that following EFSA guidelines is asking to dive into the PFAS topic on vegetables related matrices, with specific focus for wine and beer.
On January 2024 DGR 1676 following EFSA indication about the need of dive into matrices as wine and beer (largely consumed in Europe) Claim about the need of close the circle with PFAS investigating the various vegetables related matrices.
The initial need is about PFOA, PFOS, PFHxS, PFNA, but early studies delight a wider range of analytes of interest for wine producers.
Where they come from? Superficial water used for grow the crop?Air pollution?
This presentation starting from early studies do underline needs and questions about one of the most worrying emergency that is going to invade the food world.
We are going to present along the questions, starting points to make you able to face this topic in the most effective way and our PFAS method development tool EZLC.

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PL3

Advancements in Wine Metabolomics Research

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Keywords: *untargeted analysis, mass spectrometry, aging*

The metabolomics era started about 24 years ago, and wine was one of the first foodstuff subjects of analysis and investigation by this technique. Wine stands out as one of the most complex food matrices due to the diverse array of metabolites contributed by grapes, yeasts, bacteria, viticultural and oenological techniques, as well as aging processes, collectively shaping the wine metabolome. This feature made wine an excellent chemical model solution for scientists to explore the potentialities of this new technique, which enable untargeted study. Since then, metabolomics techniques were applied in several oenological studies shedding light on numerous questions from vineyard to glass.
In fact, metabolomics techniques helped us to gain knowledge on the chemical modifications occurring to the vine due to biotic and abiotic factors, to the winery during the various winemaking steps and choices, and during the aging period due to the storage and packaging parameters. This presentation will offer an overview of recent MS-based metabolomics publications investigating the behaviour of viticultural and oenological products' metabolomes under diverse experimental designs. Furthermore, it will address the organization of data and metadata according to the FAIR (Findable, Accessible, Interoperable, Reusable) principles, underscoring the importance of data management and accessibility in advancing metabolomics research in the viticultural and oenological domain.

OR18

The state-of-the-art of authentication and traceability studies on Piedmontese red and white wines: the role of lanthanides

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Keywords: *lanthanides, ICP-MS, traceability*

In today's food industry, consumers prioritize tradition, territoriality, and quality, leading regulatory bodies to refine methods ensuring food safety, authenticity, and traceability. Authentication and traceability studies serve distinct yet complementary goals: authentication distinguishes food products based on origin, variety, and production processes, while traceability establishes reliable markers linking foods to their source soil. Chemical Fingerprinting tools aid authentication, while traceability relies on diverse chemical markers like isotopes and light elements [1,2].
Recent interest in lanthanides, a group of 14 metals with similar chemical behavior, stems from their potential as geochemical markers. Lanthanides influence plant composition based on soil characteristics, serving as effective markers in simple food chains [3] but posing challenges in complex ones due to processing factors. In viticulture, wine quality hinges on grape quality shaped by factors like variety, climate, and soil composition. Wine traceability, however, faces hurdles due to processing steps altering traceability information [4].
This work discusses the role of lanthanides as possible geochemical markers in the traceability of Piedmontese red and white wines, taken as examples of wine chain of high quality. The different stages of the wine chain, i.e. soils, various root cuttings, grapes, and wines, underwent characterization by means of Inductively Coupled Plasma - Mass Spectrometry (ICP-MS), the only technique enabling the detection of lanthanides at very low concentrations. Additionally, to highlight better the existing correlations among the samples, exploratory chemometric methods were used, in particular Principal Component Analysis (PCA).
From the obtained results, it was possible to see that the profile of lanthanides remained unchanged in all the stages from soils to musts, while, as expected, fractionation occurred along the subsequent stages of the production chain. Among the possible interferers of wine traceability, it is possible to note that the winemaking process could have a significant impact on fractionation. Steps such as filtration and clarification, which involve the use of fossil flours and/or bentonites, could lead to fractionation.
In conclusion, while lanthanides offer promise as geochemical markers in wine traceability, challenges stay in their application, particularly in understanding their behavior during winemaking processes. To address this challenge, a new research project involving the Università degli Studi di Torino has just started: this project aims at evaluating how different cultivation methods, grape varieties, and winemaking processes influence lanthanide distribution.

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OR19

MS as tool for selection of a fungus-resistant Glera grape crossing (downy mildew and powdery mildew) suitable to produce Prosecco wines

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Keywords: *resistant vine varieties, Glera grape crossings, metabolomics*

In recent decades, we have witnessed a growing consumer attention and preference for natural products obtained through practices that prioritize human well-being and environmental sustainability.
Grapes and their products have also received this attention. Several grape varieties have been selectively bred to naturally withstand downy mildew and powdery mildew, which commonly afflict traditional grape cultivars [1].
Currently, a project is in progress to cultivate Glera vine clones with heightened resistance to these diseases specifically for Prosecco wine production. These clones are offspring of crosses between Glera and fungus-resistant vine varieties such as Bronner, Kunleany, and Solaris, with the dual purpose of maintaining principal traits of Glera and reinforcing its defence mechanisms against fungal diseases. Glera, known for its semi-aromatic character, is abundant in terpenols like geraniol and terpene diols [2] and lends Prosecco wines their refined citrus and floral nuances [3].
The metabolomic part of this research focused on the study of grape aroma compounds and their glycosylated precursors which directly influence the organoleptic qualities of wines [4]. These compounds serve as varietal markers, indicating traits inherited from parent vines and aiding in the selection of clones that preserve aromatic characteristics of Glera. Aromatic precursor profiles from grapes harvested in 2020, 2021, and 2023, resulting from crosses between Glera and resistant varieties were examined and compared. Among the samples studied, a Glera×Solaris crossing showed to preserve the aroma characteristics of Glera and resulted to satisfy the project aims.

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OR20

Sequential harvest of *Vitis vinifera* L. Mouhtaro grapes: influence in aromatic and sensorial characteristics

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Keywords: *Mouhtaro, volatile compounds, sensory profile*

Viticulture and the art of wine production have been an integral and essential aspect of the Greek national heritage for a more than four millennia. The progress in grape aromas and flavor precursors during berry ripening has gained attention from researchers in the last years [1]. Wine aroma is crucial for wine quality, but the mentioned attributes do not provide objective information on grape or wine aromatic potential. The purpose of the current research is to study how grape maturity of a rare and indigenous grapevine variety could influence chemical and sensorial quality of the produced wines (10, 13.4 and 15.5 v/v %) at three stages of grape ripening. The experiment was conducted in a commercial vineyard planted with Vitis vinefera L. Mouhtaro, vines more than 15 years old, located in a narrow valley named Muses valley in Askri Viotias [2]. Sequential harvests were carried out based on the physiological indicator berry sugar accumulation (baume). The wines produced with the same vinification protocol [2] were evaluated using the descriptive sensory analysis method while they underwent profiling of volatile compounds by gas chromatography-mass spectrometry (GC-MS) [3] as well as anthocyanin concentration using HPLC. Volatile and sensory profiles of the experimental wines separated significantly the three different maturities and confirmed that differences were mainly due to the grape maturity level. The concentrations of total esters show an increase during ripening as well as the same trend was recorded for the total acids and terpenes. Regarding the sensory profile, the wines that produced from the third maturity level, were characterized by higher aromatic intensity and by the descriptors such as red/black fruits, spices and caramel with a statistically significant difference from the rest of the wines. To the best of our understanding, this study represents the initial examination of Mouhtaro wines in terms of establishing a unique profile that comprises both chemical composition and sensory aroma attributes.

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OR21

Study of aromatic and nutraceutical potential of six PIWI resistant vine varieties by high-resolution MS metabolomics

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Keywords: *PIWI grapes, resistant vines, QTOF*

Rising of temperatures, droughts, increased storms, floods as consequence of climate changes create concerns for current agricultural productions. In viticulture, control of pathogens and water supply represent the manly problems linked to global warming. In the last century, many vine varieties characterized by high tolerance to fungal diseases (PIWI varieties) were produced by crossing *V. vinifera* and American and Asian *Vitis* species. Due to their attitude also to produce high-quality wines, some of them can be particularly useful to increase sustainability in viticulture and to respond to climate challenges [1].
Oenological potential of six main PIWI grape varieties was studied. In particular, the aroma precursors and colour and antioxidant compounds of grapes from four white berry *cvs* (Bronner, Johanniter, Sauvignon Rythos, and Souvignier Gris) and two red berry *cvs* (Cabernet Cortis and Cabernet Volos) were characterized by high-resolution mass spectrometry (UHPLC/QTOF).
Between 350-400 signals of putative secondary metabolites were identified, which include the profiles of glycosidic terpenols and C13-norisoprenoids (aroma precursors), flavonols, flavan-3-ols and proanthocyanidins, stilbenoids, red grapes anthocyanins. The metabolite profiles are characteristic of each variety and determine the sensorial, antioxidant, and nutraceutical characteristics of wines which can be produced.
In general, these grapes resulted rich in polyphenols (in red grapes, up to 32 anthocyanins were identified including monoglucosides and diglucosides) which make them suitable for production of wines with high antioxidant potential and suitable for aging.

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OR22

LC-HRMS and ^{1}H-NMR metabolomics data fusion as a tool for the comprehensive characterization of wine: A case study of Amarone

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Keywords: *wine profiling, multi-omics, data fusion*

In this work, both unsupervised and supervised multi-omics data fusion approaches based on liquid chromatography coupled with high-Resolution mass spectrometry (LC-HRMS) and ^{1}H-NMR spectroscopy were evaluated to comprehensively characterize Amarone wine, as a case study. In the framework of realistic Amarone integrity purposes, wine samples with different withering times (i.e., four different time points) and yeasts used for wine fermentation (namely *Inverno 1936* based on Saccharomyces cerevisiae, and *Vulcano* based on Saccharomyces cerevisiae var. bayanus) were considered. Raw datasets were fused by using multiple co-inertia analysis (MCIA) algorithm as unsupervised tool together with supervised sparse Partial Least Squares Discriminant Analysis (sPLS-DA). In this regard, the outputs given by MCIA highlighted the significant impact of withering time in describing wine samples and revealed the complementarity of the information across the two datasets (correlation RV-score of 16.4%), supporting the further application of the supervised sPLS-DA method for both factors investigated. The integrated sPLS-DA model based on withering achieved a lower error rate (6.1%) compared to yeast-based classification (10.55%). In particular, accumulations of amino acids, monosaccharides, and polyphenols have been observed as the most representative compounds based on the degree of grape withering progression. Instead, wines fermented with Vulcano yeast were enriched of flavonoids, organic acids and alcohols, with some differences that were depicted also in the amino acid composition. Overall, this approach showed the feasibility of untargeted metabolomics in predicting the chemical fingerprint of Amarone wine and future works based on multi-omics appear to be of great interest regarding the topics of wine authenticity, integrity, and traceability issues.

OR23

Variations in sensorially-relevant metabolites and indices in PDO wines of common genetic background: a case study on commercial Lambrusco wines

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Keywords: *Lambrusco, Sparkling red wines, Charmat, Champenoise*

Protected Designation of Origins (PDOs) are regulatory tools created for the enhancement of rural areas, primarily from an economic point of view. Their assumption is that it is possible to link certain characteristics of the product, mainly sensory, to its geographical origin (Barker, 2005). While the existence of specific sensory features associated with different PDOs is well established, their chemical bases are, in many cases, unknown. Lambrusco is a sparkling red wine widely produced in Northern Italy. According to National Catalogue of Vine Varieties there are thirteen Lambrusco Varieties, with which to date are produced seven PDOs wines, and three of them are located in the Modena area: "Lambrusco Salamino di Santa Croce" (Salamino), "Lambrusco Grasparossa di Castelvetro" (Grasparossa) and "Lambrusco di Sorbara" (Sorbara) (MASAF, 2022). The first difference between these Lambrusco PDOs lies in the use of different varieties. Within the intricate taxonomy of Lambrusco, Sorbara and Salamino are reported to be genetically related, while Grasparossa as genetically distant, distinguished also by and ampelographic characteristics (Boccacci, et al. 2005). A further difference among PDOs is associated with the geographic origin of the grapes, since they come from different subregions within the Modena area. This study has two main objectives: first to highlight the metabolic differences underlying the sensory diversity between different Lambrusco appellations. Second, to evaluate to which extent technological variables such as production technique (Charmat and Classico methods) and vintages impact the volatile profile of the wines. For this purpose, forty commercial Lambrusco wines from three different appellations (Salamino, Grasparossa and Sorbara), vintages and production techniques have been studied. 71 Volatile compounds belonging to the chemical classes of terpenoids, norisoprenoids, benzenoids, low molecular weight sulfur compounds, esters, fatty acids, higher alcohols and C_6 alcohols were analysed by GC–MS coupled with different extraction techniques (SPE and SPME). Comparison of Lambrusco volatiles profile with other commercial samples of Italian white sparkling and still red wines highlighted specific features of Lambrusco such as high content of acetate esters, *trans*-3-hexen-1-ol and 1,8-cineole. Different Lambrusco PDOs, differed for volatile metabolites such as linear, cyclic and bicyclic terpenes, volatile sulphur compounds, C_6 alcohols and TDN. Sorbara was found to be richer in 1,8-cineole, TDN and methyl vanillate, Grasparossa in cyclic terpenes and benzenoids while Salamino in linear terpenes and C_6 alcohols. In addition, Sorbara showed lower levels of sulfur compounds compared to the other two appellations. An association between TDN content and cineoles patterns and wine pH have been observed. The assessment of technological variables, aging treatment, and production techniques has revealed the main impact on volatile profile variability was exerted by the aging treatment itself while differences related to the production method did not reveal without adequate aging treatments.

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OR24

Study of anthocyanin evolution of a PIWI red wine undergone to oxidative conditions

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Keywords: *PIWI wine, diglucoside anthocyanins, Cabernet Cortis*

Needing of reducing use of pesticides in viticulture is increasing the interest in wines produced using fungal-resistant grapevine varieties which are characterized by relevant contents of both monoglucoside and diglucoside anthocyanins. Aging in wooden barrels induces oxygen permeation into wine but little is known on diglucoside anthocyanins evolution. Cabernet Cortis wine was subjected to addition of oxygen and oak chips and the anthocyanins changes were followed for one month. Decreases of 90% total monoglucosides, 80% acylated monoglucosides, 65% diglucosides and 90% acylated diglucosides were observed. Monoglucosides form pyranoanthocyanins and the lower steric hindrance has favorited their polymerization with flavanols. Instead, decrease of diglucosides resulted correlated to the number of B-ring hydroxyl groups by inferring a preponderant aglycone oxidation. However, three flavonol-anthocyanin-diglucoside derivatives named (epi)catechin-ethyl-Mv-dihexoside, (epi)catechin-ethyl-Pn-dihexoside, and (epi)catechin-Mv-dihexoside A-type were identified in wine for the first time. Findings of research are useful for tuning of suitable oenological practices to stabilize the color of these wines (type of barrel, aging times, oxygenation practices) and lower the malvin content which currently is recommended by OIV at maximum 15 mg/L and is critical issue for their commercialization.

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Short Presentations

SP1

Volatile profile and antioxidant properties of Ruché wine from Piedmont

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Keywords: *GC-MS, HS-SPME, wine volatile components*

The characterization of a typical red wine from Piedmont (north-west Italy), Ruché, was performed by means of headspace solid phase microextraction[1] coupled to gas chromatography–mass spectrometry and UV-vis spectroscopy. The main volatile compounds of the wines were identified and semi-quantified by GC-MS. Based on these results, Principal components analysis (PCA) was performed in order to identify possible clusters among the different samples analyzed. Ruché is a red wine: as such, its antioxidant power is expected to be certainly higher than that of rosé or white wines. Experiments were then carried out in UV-vis spectrophotometry: inhibition of the signal (λ_{max}=517 nm) of the DPPH model radical as the effect of different incubation times with wine was monitored.

Fig. 1: Ruché wine production area

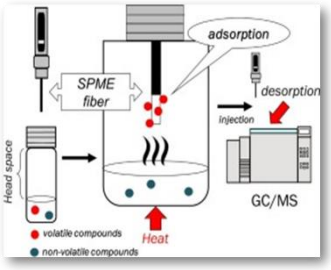
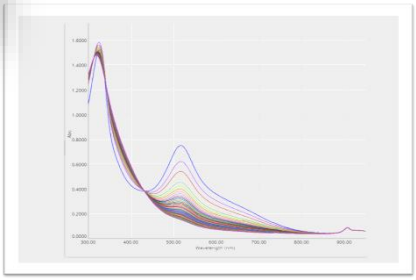


Fig.2: HS SPME design



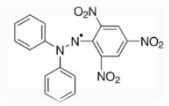


Fig. 3: kinetics of DPPH consumption in wine

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SP2

Effect of storage time and temperature on the volatile composition of Malvasia di Candia Aromatica white wine by GC×GC-MS

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Keywords: *HS-SPME-GC×GC-MS, vine vigour, aromatic varieties*

The within-variability of the vineyard affects the grape composition, including sugars, acids, aroma compounds, and polyphenols; therefore, it strongly influences the sensory and chemical characteristics of the wines [1]. In this scenario, precision agriculture techniques and selective harvesting are powerful tools to manage vineyard variability and to enhance the sustainability of the winemaking process and the quality of wines [2]. This study aimed to assess the effect of vine vigor (high and low), time, and storage temperature on the aroma profile of Malvasia di Candia Aromatica white wines from Piacenza's province hills.
In our trial, the vineyard vigor map was obtained through MECS-VINE sensor [3], then translated into a prescription map with which the grapes were harvested by a vigour-selective mechanical harvester (NH Braud 9080L with Enocontrol system, Italy). For each vigour zone (Low Vigor, LV; High Vigor, HV; Control, C) 30 kg of grape were collected, immediately pressed and fermented at 14°C. At the end of alcoholic fermentation, the wines were bottled in dark glass and stored for 6 months at 5°C; 15°C and 25°C. The wines were sampled at 0 days (T_0), 90 days (T_1) and 180 (T_2) days and the volatile organic compounds (VOCs) were analyzed by HS-SPME-GC×GC-qMS (Shimadzu, Italy) equipped with a ^{1}D SLB-5ms apolar column and a ^{2}D SupelcoWAX polar capillary column. VOCs were identified by matching with NIST20 libraries and the linear retention index. The data analysis (ANOVA, PLS-DA, Fold Change Analysis) was performed by MetaboAnalyst platform. A pool of 112 volatile compounds was quantified in experimental wines, among these: 63 terpenes, 5 norisoprenoids, 34 esters, and 5 higher alcohols. At T_0, the LV wines showed higher concentrations of terpenes compared to HV and C wines. The most relevant compounds in terms of concentration were Linalool, Geraniol, and L-α-Terpineol that were detected at significantly higher concentrations in LV. In order to evaluate the effect of time and temperature of storage the dataset was run with a PLS-DA statistical model, the variable importance in projection (VIP) analysis was used, and then coupled with Fold Change analysis (FC). For the storage time, the most characterizing compounds were mainly terpenes that were mainly down-accumulated over time (e.g., trans-Geranylacetone, trans-Nerolidol, α-Pinene, trans-2-Pinanol, and Linalool). On the other hand, for storage temperature, more heterogeneous results were obtained: both terpenes and esters characterized the modification of the aromatic profile and were both down- and up-regulated. Among terpenes, a general reduction of Linalool and Geraniol, and the increase of their oxidated and derivates forms were detected (e.g., Linalool 3,7-oxide, Nerol oxide). Relevant up-regulation were also detected in charge of TDN and TPB,1. The obtained preliminary data confirmed the expected differences in aroma composition among wines coming from different vigour zone, especially for the differences highlighted by terpenes [2]. According to the data, the aroma compounds follow literature available patterns, especially when stored for a long time at higher temperatures [4].

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SP3

Base wines for sparkling wine from resistant varieties: effect of the altitude on the aromatic profile

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Keywords: *Resistant varieties, aroma, base wine*

In a recent context where consumers pay an increasing attention to sustainability in the decision-making process, the use of the resistant varieties in the wine sector have returned at the forefront. Not only but the use of these varieties would reduce production costs due to the lower pesticide utilization to control grapevine moulds. However, the specific aroma profile looked for in base wines is strongly influenced by the cultivar.
This work aims to study the volatile composition of base wines produced from five resistant varieties (Bronner, Solaris, Johanniter, Souvignier Gris, Vinera) cultivated in two experimental vineyards located in Trentino (IT): one situated on the valley bottom and the other on the hillside. The results were compared with those of Chardonnay, the main variety used in this area nowadays for sparkling wine production, cultivated in the same plots. The volatiles were extracted from the base wines and the GC-MS/MS analysis [1] allowed to quantify the aromatic compounds belonging to six different chemical classes: acetates, ethyl esters, alcohols, fatty acids, terpenes and norisoprenoids.
Among the varieties, Souvignier Gris was characterised by methyl salicylate and 1-hexanol, while Solaris stood out for the concentration of β-damascone, acetates and ethyl esters. Bronner showed significant contents of some grape-derived metabolites, such as β-damascone and linalool. This terpene was also present in higher quantities in Solaris and Johanniter. Regarding the location, acetates and ethyl esters were higher in base wines of the valley bottom and fatty acids, higher alcohols, and terpenes in the hilly plot wines.

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SP4

Aroma characterization of commercial Prosecco sparkling wines

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Keywords: *Prosecco, volatile compounds, GC-MS*

The typicality of a wine, as well as its aromatic identity, are attributes highly sought after and requested by the current market. It is therefore of considerable technological interest to delve deeper into the aromatic aspects of specific wines and to identify the odorous substances involved. Many factors influence the volatile chemical profile of sparkling wines including production method, grape variety, base wine production method, grape origin, yeast strain, aging periods, and lees contact. Prosecco wines are described with aromatic notes of peach, pear, wisteria, flowers, ripe fruit, citrus, spices and sage, with differences due to the sub-areas of grape production. As regards the chemical composition of Prosecco wine, it still remains little studied, few works have studied the profile of the volatile compounds responsible for its olfactory bouquet.
The main objective of the study was the characterization of the aromatic profile of Prosecco sparkling wines belonging to the DOCG Valdobbiadene, DOGC Asolo and DOC Treviso, evaluating the presence of typical characteristics attributable to the origin. Another objective was to evaluate the existence of sensory and chemical spaces in the sampled wines.
Low molecular weight sulfur compounds and terpenoids, were analyzed by SPME-GC/MS using two distinctive methods. Fermentative volatile compounds were extracted using SPE and then analyzed by GC-MS.
The aroma of Prosecco wines was mainly characterized by ethyl esters of straight-chain fatty acids and acetates. Other compounds that characterized the volatile composition of Prosecco wine were fermentative compounds such as fatty acids, phenylethyl alcohol and the sulfur compounds dimethyl trisulfide and dimethyl sulfide. Monoterpenes could participate in the floral notes in some samples, however in general their concentration remained below their olfactory threshold. Compositional differences were observed between the three PDOs analyzed with Asolo wines generally richer in terpenes, norisoprenoids and sulfur compounds. Valdobbiadene was characterized mainly by benzenoids, while Treviso by hexanoic acid and phenylethyl alcohol. The sensorial analysis carried out using a sorting methodology indicated that the wines were grouped to form two bunches that did not perfectly correspond to the 3 PDOs considered. The two clusters were characterized by 22 compounds, in particular cluster A was characterized by a higher ester content while cluster B was more distinguished by higher levels of VSC, cyclic terpenes and TDN.

SP5

New Approaches to Traceability of Oenological Tannin Origin: High-Resolution Mass Suspect Screening Study

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Keywords: *tannins, botanical origin, Orbitrap*

Tannins have long been used by winemakers, but in the last twenty years, they have undergone a profound evolution in industrial preparation and commercial formulation, expanding their specific technical applications [1]. They are extracted from many plant species and chemically belong to the polyphenol family [2]. Among the different types of tannins, some are naturally present in must and wine, while others can be added as adjuncts to achieve various objectives such as must and wine protein stabilization, color stabilization, or improved organoleptic properties. In particular, the OIV identifies four categories of tannin from a chemical-compositional perspective: procyanidins/prodelphinidins (grape tannins), profisetinidins/prorobinetinidins (acacia and quebracho tannins), gallotannins, and ellagitannins (oak, chestnut, and oak tannins). Each category has different enological characteristics and a different market cost.
Thanks to the recent availability of new advanced analytical techniques, in 2022, the OIV defined, through new monographs dedicated to enological tannins (OIV-OENO RESOLUTION 624-2022), new provisions for the analytical confirmation of their botanical class membership.
In this work, an analytical method was developed using high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) for the analysis of condensed and hydrolyzable tannins. This allowed the characterization, also through suspect screening approaches, of the various monomeric and oligomeric components specific to each botanical class, allowing quantification, as catechin or gallic acid, respectively.
The compositional characterization of a wide selection of enological tannins (N=53) sampled from the market, whose compliance with the OIV regulations in force until 2022 had already been positively verified, was then carried out with the aim of verifying the reliability of their botanical classification through the new guidelines.

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SP6

Evaluation of oenological strategies for the valorisation of methyl salicylate in Lugana wines

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Keywords: *Methyl Saliciyate, mixed fermentation, Lugana wines*

Lugana wines are characterized by high levels of methyl salicylate (MeSA) [1,2]. This compound has a distinctive minty wintergreen aroma [3,4]. However, MeSA in grapes and musts exists mainly in the form of a non-odorous glycosidic precursor. For the winemaker, it becomes important to understand how to exploit this aromatic potential in order to express varietal olfactory characteristics. For this reason, we decided to investigate the effect of 3 different enological strategies. The first aim was to evaluate the impact of mixed fermentations with Saccharomyces and non-Saccharomyces yeasts on MeSA concentrations in the wines obtained. In addition, the impact of products normally used for wine stabilization, such as activated carbon, bentonite, PVPP and plant proteins, was evaluated. Finally, the impact of enzyme products was also evaluated. Volatile composition of wine was analysed by HS-SPME-GC-MS.
Results show that mixed fermentations did not lead to significant differences in methyl salicylate content, although it appears that *Hanseniaspora vineae* has a greater capacity to release the molecule. Regarding treatments with different oenological products, we noticed that the use of charcoal leads to a drastic decrease in methyl salicylate concentrations probably due to, bentonite has no significant effect on its concentration; surprisingly PVPP and vegetable proteins cause an increase of free MeSA. With regard to enzyme products, an general increase in methyl salicylate concentration was noted, but only one product resulted in significant differences compared to the control.

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SP7

Pathways to cineole formation in aged Amarone wines

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Keywords: *Amarone wine, cineoles, wine aging*

Amarone della Valpolicella is an Italian red wine elaborated from partially withered grapes. These varieties have been previously shown to provide wines with a relatively high content of terpene compounds, prone to several acid-catalyzed reactions at wine pH [1,2]. The occurrence of terpenes in wines from non-aromatic grapes is associated with chemical and biochemical transformations of odorless precursors originally present in the grape, mostly glycosidic non-volatile compounds that can be released either by enzymatic or chemical hydrolysis happening during aging [3,4,5]. Low pH can promote the hydrolysis of these precursors and catalyze cyclization reactions, leading to a complex pool of terpenic compounds [1]. Among these compounds are the bicyclic terpenes 1,4-cineole and 1,8-cineole, typically occurring in aged Corvina wines [1,2]. Previous work suggested that these compounds can form from glycosylated terpenes as well as from free terpenes, among which terpinene-4-ol has a prevalence in the case of Amarone. This work aimed to investigate the role of different possible precursors on cineole formation during Amarone aging in model conditions. Two main hypotheses were investigated: 1) terpinen-4-ol transformation to 1,4-cineole, where model wine at different pH (2.9, 3.3, 3.6) was spiked with terpinen-4-ol and submitted to accelerated aging; 2) 1,4-cineole formation from glycosidic precursors, where the bound fraction from Amarone experimental wines was submitted to accelerated aging at different pH (2.9, 3.3, 3.6). For both experiments, samples were placed at 60 °C for 1 week and 1 month and analyzed by HS-SPME/GC−MS.

For experiment 1, a good correlation was found between 1,4-cineole and terpinen-4-ol with an R_2=0.8701. Regarding the influence of pH (1 month of aging), 1,4-cineole was released significantly higher at pH 2.9 compared to higher pH (3.3 and 3.6), around 1.6 and 2.2 times, respectively. Results from experiment 2 showed that the occurrence of 1,4-cineole and 1,8-cineole from glycosidically bound precursors is considerably low, with concentrations going up to 0.04 and 0.06 μg/L, respectively (after one month of aging). Regarding pH, no differences could be observed in the levels of cineoles for samples at 1 week. Instead, at 1 month, significant differences were found for samples at low pH (2.9). These results are in accordance with what was observed in experiment 1. However, the levels found are considerably lower.

In conclusion, results showed that 1,4-cineole is well correlated with terpinen-4-ol during aging. This is of interest due to the high levels of terpinen-4-ol in Amarone wines and terpinen-4-ol origin, which is associated with withering, a key process of Amarone production. The development of terpinen-4-ol to 1,4-cineole can suggest them as potential markers of Amarone. Additionally, pH was an important modulator in the release of 1,4-cineole, wines with lower pH showed higher levels of cineoles. Furthermore, glycosidically bound terpenes found in Amarone experimental wines submitted to accelerated aging show significantly low levels of cineoles, indicating that their occurrence is associated with the free fraction of terpenes in wines.

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Restaurants and Osterias

Restaurants

Il Brillo Parlante is an enoteca and restaurant located in Asti, offering a selection of fine wines and a menu that features both Italian and international cuisine.
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Address: Via Teatro Alfieri, 5, 14100 Asti, Italy

Osteria del Diavolo: Osteria del Diavolo is a highly recommended dining spot in Asti, known for its rustic ambiance and traditional recipes. Located at Piazza San Martino, the restaurant is celebrated for its attentive service and a menu rich with local specialties, making it a beloved choice among residents and visitors alike.
Address: Piazza S. Martino, 6, 14100 Asti, Italy; T: 0141 30221

Caffè Mazzetti: Caffetteria Mazzetti, located in Asti, is more than just a coffee shop; it's a cocktail bar and restaurant that pays homage to the rich culinary traditions of the region. The menu is crafted with love and passion, offering classic flavors in a sophisticated atmosphere. This establishment is ideal for those who appreciate fine coffee and dining in a historically significant setting.
Address: Corso Vittorio Alfieri 357, 14100 Asti, Italy; T: 0141 324299

Collina delle Balene: specializes in seafood, offering a fixed menu that emphasizes quality and variety. It's the perfect destination for those looking to enjoy exquisite fish dishes without traveling to the coast.
Address: Via Fontana 13, 14100 Asti, Italy; +39 3920695736

Pastificio Cit ma Bon, specializes in offering fresh, handcrafted pasta. The establishment is renowned for its dedication to traditional pasta-making techniques, ensuring authentic Italian flavors that resonate with both locals and visitors.
Address: Via Garetti, 29, 14100 Asti, Italy; T: +39 349 289 6400

Il Cavallino Alato: Il Cavallino Alato in Asti operates as a combination of a tavern and birrabar, offering a diverse menu that includes both Italian and international specialties, alongside a wide selection of craft beers and Piedmontese wines.
Address: Via Quintino Sella, 3/1, 14100 Asti, Italy; T: +39 349 316 8081

Casa Dirce is a charming restaurant located in the heart of Asti, renowned for its traditional Piedmontese cuisine. This establishment offers a cozy dining experience, emphasizing local ingredients and classic recipes.
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Ristorante Angolo del Beato, located in the charming town of Asti in Piedmont, offers a delightful dining experience with a focus on traditional Italian cuisine. Nestled in a picturesque setting, this restaurant is praised for its authentic dishes that showcase the flavors of the region.
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Address: Corso Vittorio Alfieri, 149, 14100 Asti, Italy

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