



1st Science & Wine World Congress

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Science & Wine
THE WINE OF THE FUTURE

Presentation

Science & Wine 2019 follows the success of the first National Conference in 2017, also held in Porto, and will continue to address the multitude of issues associated with wine technology, chemistry and biochemistry, health and environmentally sustainable development of wine industry. The topic chosen for this 1st Science & Wine World Congress is “The Wine of the Future”. This is the moment to bring together the experience of experts from industry, academia, research institutions, service providers, regulatory agencies and policy makers and design the wine that will be consumed in the future. To do this, it is important to understand how new technological advances could be used towards to wine production increase and wine quality improvement. Wine authenticity, wine health effects and sustainable practices of wine production are issues considered by consumers in their purchasing processes, therefore it is also important to discuss them when designing the wine of the future.

Track 1: Technology for wine analysis

- What type of wine do you want to drink? The nanotechnology engineered wine.
- Could portable electronic tongue replace human panelists to measure toxic substances and make objective analysis in wine?
- Could electronic tongue be used as analytical tool dedicated to qualitative and quantitative assessment of wine production?
- Is really needed to develop a simple device that can mimic the human sense of smell to be used in routine industrial applications?

Track 2: New technological trends in wine production

- Which are the novel processing applications to improve wine quality characteristics?
- The inevitable application of big data to wine production!
- Recent advances in precision viticulture and robotics applied to the vineyard.

Track 3: The consumers of the future

- Drink or not drink wine? A science communication question?
- Is the reduction of the SO₂ amount in wines a decisive strategy for the wine industry?
- Does supplying organic wines enhance a firm's brand image?
- Consumer perceptions of luxury wines: the example of ice wines.
- There is a future for wines like the blue ones?
- The impact of wine on aging and longevity

Track 4: Sustainable viticulture

- Use of different vine species and varieties in viticulture: be prepared to climate change.
- Sustainable viticulture: water quality/treatment, viticulture waste, and energy-related issues.
- What is the wine's carbon footprint size, and how can technology reduce it?

Track 5: Fate and biotransformation of wine residues industry

- Use of wine by-products in animal nutrition and in agriculture.
- What's new in biopotential of wine by-products applied in the pharmaceutical industry?

Track 6: Future strategies for wine traceability and authenticity

- Recent advances in fingerprinting techniques in wine authenticity and traceability
- Have DNA-methodologies potential to be applied for wine authenticity purposes?

Paula Cristina Paulo Videira da Silva

President of Science & Wine 2019

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Invited Speakers



Nanotechnology in Wine: Emerging technologies of fermentation

Antonio Morata Technical University of Madrid (UPM), Spain



Live scale is nanometric, in this range of size most of cell structures are working: membranes, proteins, nucleic acids, biopolymers. At nanoscale, interactions are promoted, surface phenomena are enhanced, and catalytic and synergistic processes are developed. Nanotechnology opens the door to new high effective and sensible sensors, new materials with unbelievable properties, antimicrobial nanopolymers and new ways of interaction among the nanostructures and cells. In wine technology nano-applications let us cheaper and sensible sensors, new antimicrobial compounds, and the improvement of processing techniques. The use of other emerging technologies as high pressure technologies (HHP and UHPH), pulsed electric fields, irradiation (pulsed light, UV, beta-irradiation) help us to process the grape faster, increasing phenolic and aromatic extraction, and simultaneously reducing wild grape microorganisms. The reduction of wild grape microbioma facilitates the use of new fermentation biotechnologies like the use of non-Saccharomyces yeasts and yeast bacteria coinoculations. Also helps to moderate the use of sulfites in enology.



Wine and health: a matter of moderation?

Celestino Santos-Buelga University of Salamanca, Spain

It is beyond doubt that alcohol is harmful, and that its irresponsible and excessive consumption has severe consequences for health and serious social implications. On the other hand, evidences accumulated in recent decades suggest that light to moderate alcohol consumption, mostly in the form of red wine, may be related with beneficial effects for health, especially with lower incidence of coronary heart disease. This has received the attention of the media, which have contributed to spread a message, sometimes confusing, contradictory or spurious, about putative health benefits of drinking. In the presentation, an outline on the available knowledge on wine and health relationships will be made, paying particular attention to the role of wine polyphenols and the patterns of alcohol consumption on the putative beneficial effects.

Wine tartaric stabilization: what options are there for replacing the cold stabilization method?

Encarna Gomez University of Murcia, Spain



The tartaric stabilization of wines before bottling to avoid the precipitation of tartaric acid salts is an important and common step during wine production and cold stabilization, an effective but costly process, has been commonly used in wineries for eliminating the excess of instable tartaric salts. Other techniques are now taken into consideration for replacing the use of low temperatures. Two of these techniques, ion exchange resins and electro dialysis, involve the reduction of the ions causing the presence of precipitated tartaric salts. Other techniques, such as the use of carboxymethylcellulose and potassium polyaspartate, are based in the formation of protective colloids or the inhibition of the crystallization of salts. The effect of these technologies applied in white and red wines as regards not only stabilization but also their effect on chromatic and sensory characteristics will be discussed.



Stable isotope ratio analysis of wine: new prospective!

Federica Camin Fondazione Edmund Mach, Italy

The stable isotope ratios of H (D/H), C ($\delta^{13}\text{C}$) and O ($\delta^{18}\text{O}$) have been analysed using IRMS and SNIF-NMR in wine and must since 1987, using official standards that are listed as OIV methods. This analysis enables the detection of sugar and water addition as well as mislabelling, on the basis of a comparison of data with an official reference databank, set up according to the current European Regulation 273/2018. Recently the effect of some oenological practices, such as dealcoholisation, grape withering and the stopping of fermentation on these isotopic ratios has been investigated. The encountered variations in wine isotopic ratios have to be considered when interpreting the isotopic values of actual samples. Moreover, more innovative isotopic methods, based on the analysis of the stable isotope ratio of other elements or of other components (e.g. $\delta^{18}\text{O}$ of wine ethanol, $\delta^{13}\text{C}$ of the main higher alcohols and vanillin and $\delta^{15}\text{N}$ in must, wine and in the extracted proline) have been developed.

Application of Surface Plasmon Resonance (SPR) as a tool to study astringency measuring the interaction between tannins and salivary proteins

Fernando Zamora Universidad Rovira I Virgili, Spain



The interaction between mucin and 3 oenological tannins (ellagitannins, gallotannins and proanthocyanidins) was measured by Surface Plasmon Resonance (SPR). These tannins were analysed and their astringency was determined using the Astringency Index method and by tasting. The interaction constants were determined using a Biacore SPR device. The results indicate that the ellagitannins are more astringent than gallotannins and those, in turn, are more astringent than seed proanthocyanidins if the richness of the commercial extracts is considered. The astringency index of these tannins had high correlation and regression coefficients with their kinetic and thermodynamic dissociation constants. This data support a hypothesis that astringency depends not only on the thermodynamic tendency to form the complex between tannins and salivary proteins but also probably on the time required to dissociate the complex.



Effects of water status on vegetative growth, yield and grape quality in cv. Touriga Nacional and cv. Touriga franca in Douro region

Jorge Bernardo Lacerda de Queiroz University of Porto, Portugal

Douro Region is characterized by a typical Mediterranean climate, with schist soils and subjected to water, temperature and radiation stress conditions. In 2018, a deficit irrigation trial was conducted with field grown varieties Touriga Franca (TF) and Touriga Nacional (TN). In this experiment, grapevines were subjected to four irrigation regimes: 75% of crop evapotranspiration (ET_c) (R75); 50% of ET_c (R50); 25% of ET_c (R25); non-irrigated (R0 - Control). Water was applied bi-weekly from veraison until fifteen days prior to harvest. No significant differences were found in canopy density and total leaf area between irrigation modalities, but the values were higher in TN when compared to TF. Predawn water potential (Ψ_{pd}) showed that TN had higher stress during the season, with lower values in R0 treatment. At harvest, values of probable alcohol (except in R75) and total acidity were higher in TN with no differences between irrigation modalities. Values of pH, malic acid, anthocyanins and polyphenols were higher in TF. Statistic differences were observed in TF in probable alcohol (R0 vs R75) and in anthocyanins content (R75 vs R0 and R25). Number of clusters/plant and production/plant were higher in TF, with significant differences in number of clusters in R0 vs R50 and differences in yield in R0 comparing to R50 and R75.

Keywords: *Vitis vinifera* L., Berry composition, Crop evapotranspiration, water deficit irrigation, yield.

Bioelectronic tongue based on electrochemical sensors.

Applications in oenology



Maria Luz Rodriguez University of Valladolid, Spain

In the last years new methods for the analysis of complex liquids -the so-called electronic tongues- have been developed. They consist in arrays of sensors (usually electrochemical sensors) coupled to a pattern recognition software [1]. E-tongues analyze the sample as a whole without need of separating it in simple components. The objective of our work is to develop a bioelectronic tongue dedicated to the analysis of wines and grapes. For this purpose, arrays of sensors formed by phthalocyanines combined with biosensors (containing enzymes such as glucose oxidase or tyrosinase and phthalocyanines as electron mediators), have been developed. Sensors are tested individually towards model solutions of antioxidants or sugars usually present in wines and grapes. The electrochemical responses are characterized by complex curves that contain information about the pH and the content of sugars and antioxidants. The sensibility, detection limit and stability of the sensors has been evaluated. These experiments have been used to select the most appropriate sensors to construct the array. The final array of sensors has been used to analyze red wines and red grapes of five different varieties (From de D.O. Ribera de Duero. Spain). The sensor array coupled with pattern recognition techniques is able to distinguish the red wines and red grapes on account of their chemical nature.

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Acknowledgements

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Effect of grape /wine polyphenols in the colorectal cancer, an aged-associated pathology

Norbert Latruffe University of Burgundy, France



Since 2004, in European countries, cancer the first cause of mortality, ahead the cardiovascular pathologies. The colorectal cancer is the second most frequent cancer. Epidemiological, clinical, preclinical and experimental studies demonstrate that the beneficial effect of diet especially mediterranean diet towards digestive cancers. Fibers, polyphenols, omega-3 fatty acids would be the most protective components. Moderate wine consumption is considered to be good for health, particularly against atherosclerosis but also possibly against cancer. It is common to attribute beneficial effects to polyphenols notably resveratrol which is well known for its protective action due to - its pro-apoptotic effect on transformed cells; - its pro-differentiating mechanism - and its ability to modulate pro-oncogenic or tumor-suppressor miRNAs. The objective of our talk focused on the possible effect of wine polyphenols compared to the biological action of resveratrol. For this purpose, we evaluated the efficacy of red wine extract (RWE) and of resveratrol on colorectal established cancer cells and on intestine precancerous state.

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Wine authenticity: a multidisciplinary challenge.

Paula Martins-Lopes University of Trás-os-Montes and Alto Douro, Portugal

Wine adulteration/mislabeling is a practice that can harm economically a brand, leading to consumers trust decline. A robust wine authenticity scheme can only be achievable through a multidisciplinary approach involving both the geographical provenience and grapevine varietal identification. The talk will consist on dealing with this problematic, what are the concerns and barriers found and how they can be overcome. A multidisciplinary approach will be presented as one of the means to design a robust authenticity system. Emphases will be given to grapevine varietal identification using DNA-based methodologies.

Wine authentication using GC-IRMS approach

Matteo Perini Fondazione Edmund Mach, Italy



Since ancient times, wine has been considered one of the most sophisticated matrices. In the last decades, the continuous rise in volumes and in prices of wine has encouraged adulteration in the oenological field. One of the most commonly used techniques is the sugar addition in the form of cane, beet sugar, or syrup coming from vegetal fonts like cereals or fruits. Since 1990, the International Organisation of Vine and Wine (OIV) have issued two specific official isotopic methods to fight against this practice, but they are not always effective. With the aim to develop a new method to identify the sugar addition, we compared the ^{13}C of sugar extracted from grape must following the official method UNI ENV 12140:1997 to the ^{13}C of the amino acid proline through GC-IRMS, after extraction and derivatization of the samples. In addition, following the same approach, the carbon isotopic composition of two characteristic grape must sugars (myo and scyllo – inositol) was measured to identify the illegal correction of their concentration. We concluded that stable isotope ratio analysis using GC-IRMS approach represents a novel analytical tool to support and improve certification and control procedures.



Oral Communications

EFFECT OF MARC PRESSING ON SANGIOVESE WINE QUALITY

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Sangiovese is one of the most widespread grape cultivar in Italy. Owing to Sangiovese's ability to take on characteristics of region, climate and those imparted by the winemaker, wines made from this grape vary widely in colour and flavour. In this study we evaluated the effect of marc pressing (free run vs press wines) on polyphenolic and sensory quality of Sangiovese wines from different areas of Tuscany. 100% Sangiovese grapes were elaborated according to the same protocol obtaining 8 free run (F) and press (P) wines. Polyphenolic analyses: colour intensity, hue, CIElab coordinates, pigmented polymers, anthocyanins, tannins, BSA-tannins, total phenolics, flavans, phenolic compounds (HPLC-MS), phloroglucinolysis (mDP, %G, %P). Sensory analysis (astringency intensity and subqualities, taste, odor, aroma) was carried out by a trained jury. The effect of marc pressing differed depending on wine. In general, P wines showed higher A/T, less flavonols, more flavan-3-ols, more herbaceous, spicy and less fruity and floral aromas than F wines. Subqualities as silk, green and satin were higher in F. The colour of Sangiovese was mainly influenced by geographical area. Furthermore, wines from the same area showed a high variability. Marc pressing showed an influence on Sangiovese wines depending on the production area.

EFFECT OF THE TYPE OF CLOSURE AND BOTTLE COLOR ON THE AROMATIC QUALITY OF ALBARIÑO WINES AFTER LIGHT EXPOSURE

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Nowadays, one of the key points on the white wine preservation is controlling its storage over time. The quality of white wines is clearly affected by temperature, humidity and lighting conditions during storage. In this way, the type of closure and the color of bottles, among other parameters, are also important factors to be considered. The inadequate combination of all these factors can lead to a loss on the aromatic and colorimetric quality of wines. The main objective of this work was determining changes on the aromatic profile and color of Albariño wines exposed to light depending on the type of closure and the color of bottle. The effect on the sensory perception was also studied. The study was carried using different colors of glass bottle (clear, amber and green) and types of closures (screw cap, agglomerated cork and synthetic plant-based closures). The aromatic composition by GC-MS, the evolution of color (CIELAB parameters), and the sensory analysis were performed at 3, 6, and 12 months. The aromatic composition and color of the studied wines was clearly affected by the light exposition. In general, the screw cap and the green glass bottle seem to better preserved the quality of Albariño wines.

EMOTIONAL AND COGNITIVE REACTIONS DURING SMELL AND TASTE PHASES IN WINE EXPERIENCES

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Emotional engagement and cognitive interest are two crucial indexes that provide information about consumer decision-making processes. In the competitive environments in which wine manufactures operate those indexes should be studied in order to offer better insights about individual preferences during a wine experience. In this context, neuromarketing sheds light on non-conscious human reactions. Different neurophysiological measures such as electroencephalogram (EEG), galvanic skin response (GSR) and heart rate (HR) are applied, together with the traditional marketing method of self-report. Different case studies with both wine expert and non-expert participants will report the different perceptions of wine during its degustation, particularly during the smell and taste phases. Results show differences between wines and also differences in human brain perception between smell and taste phases. It is confirmed how, considering the intrinsic features of wine, the smell phase involves more emotional engagement, unlike the taste phase, which involves more cognitive interest. These results give wine manufactures the possibility to improve the quality of their product. Neuromarketing applications also enable an analysis of the extrinsic properties of wine, such as label or prices, in order to develop marketing strategies.

ANALYSING THE IMPACT OF MUSIC ON THE PERCEPTION OF RED WINE VIA TEMPORAL DOMINANCE OF SENSATIONS

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Several recent studies, such as [1], examined the impact of music on the evaluation of food and drink, but few have relied on time-based methods. Given that both music and the consumer experience of food/drink are typically time-varying in nature, we consider temporality in this study. 39 participants evaluated a Pinot Noir using Temporal Dominance of Sensations (TDS) methodology with 8 attributes (red fruit, tannins, alcohol, woody, sweet, acidic, spicy, and bitter). The wine was presented in 3 different music conditions: 1) no music, 2) while listening to Brian Eno's "Discreet Music", 3) while listening to Mussorgsky's "Night on the Bald Mountain". Onset of acidity is earlier in the silent condition as compared to either of the soundtrack conditions, and astringency and alcohol are less noticeable with music. The temporal order of bitterness and acidity prominence changes depending on the soundtrack. Tasting wine while listening to different soundtracks leads to different perceptions of dominant flavours.

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IDENTIFICATION CHARACTERIZATION OF WILD SACCHAROMYCES AND NON-SACCHAROMYCES YEASTS FROM MUST AND WINE OF GRAPE VARIETIES IN TURKEY FOR THE DEVELOPMENT OF NEW STRAINS FOR WINE PRODUCTION

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Indigenous yeast strains of spontaneous wine indicate regional differences in their characteristics and play important role in authenticity due to their specific metabolites. The aim of this study is to isolate, identify and characterize yeast isolates of grape musts and wine produced from unique grape varieties of Turkey. In this study, 300 *Saccharomyces cerevisiae* and 103 non-*Saccharomyces* yeasts from must and spontaneously fermented wines of 4 red, 1 white grape types of 3 wine regions in Turkey were isolated. Ethanol sulfite agar and Lysine agar with biphenyl were used for isolation of strains of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts respectively. Isolates were analyzed by sequencing analysis of 5.8S-ITS region and/or D1/D2 domains of the large subunit ribosomal RNA genes. Selected *Saccharomyces cerevisiae* strains were also genetically analyzed using karyotyping using PFGE [Pulse Field Gel Electrophoresis]. The sequence analysis assigned 103 non-*Saccharomyces* isolates as non-*Saccharomyces* species of *Metschikowia*, *Lanchancea*, *Hanseniaspora*, *Rhodotorula*, *Starmarella* and selected *Saccharomyces* isolates as *Saccharomyces cerevisiae*. 3 different chromosome patterns were obtained by karyotyping. New strains of *Saccharomyces* and non-*Saccharomyces* were identified and characterized for wine making.

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IMMUNE SYSTEM: A ESSENTIAL KEY TO UNDERSTAND THE CHEMOPREVENTIVE ACTION OF RED WINE EXTRACT AND RESVERATROL AGAINST COLORECTAL CANCER

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Lymphocytes Th17 play a crucial role in the immune response of the host to cancer and present inflammatory properties through their ability to produce interleukins i.e. IL-17. In this study, we highlights for the first time the key role of Th17 lymphocytes in the action of resveratrol and red wine extract (RWE) to counteract colorectal cancer progression and metastasis. T cells were isolated from the lymph node and mouse spleen, and were differentiated in vitro in Th17 cells in the presence of RWE. At the end of treatment, we determined the pro-inflammatory IL-17 by ELISA method and the key actors controlling the differentiation especially Sirt-1 and RORc. Moreover, tumor growth monitoring studies were carried out in mice invalidated for IL17 as well as transgenic mice with conditional invalidation of Sirt-1 in CD4 T cells by crossing Cre-CD4 mice with Flox-Sirt-one mice. RWE and resveratrol decrease Th17 differentiation and pro-inflammatory interleukins production. These events are associated with a decrease of tumor growth and angiogenesis in an IL-17-dependent manner. These protective effects are dependent of a NAD-dependent deacetylase sirtuin-1. RWE and resveratrol used the modulation of specific immune Th17 cells to counteract colon cancer progression.

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SOLID PHASE EXTRACTION APPLIED TO HPLC-HDX-HRMS/MS TO UNRAVEL A WHOLE SERIES OF UNCONVENTIONAL PROANTHOCYANIDINS FROM WINE AND OTHER PLANT FOODS

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A tetrameric procyanidin characterized by an unusual B-type 'crown' (cyclic) structure was recently identified in wine.¹⁻³ The presence of other 'crown' proanthocyanidins (PAC) congeners with various monomers composition and conformations was also hypothesized. These PAC display higher polarities than their non-cyclic analogues and also masses compatible with the isomeric A-type PAC. The latter ones have already been found in wine, although they are known to be more common in cranberries (*Vaccinium* sp.) and peanut skins (*Arachis* sp.). An optimized approach based on a C18 SPE purification was applied to hydrogen/deuterium exchange (HDX) HPLC-HRMS/MS to characterize these PAC in wine and other plant foods. The study enabled to identify a whole series of congeners (procyanidins and prodelphinidins) in wine that cannot be assigned to A-type PAC, but instead to B-type 'crown' (cyclic) structures. The proposed approach allowed the identification of a series of unconventional proanthocyanidins (from tetramers to hexamers) in wine, with various substitutions. Their promising role as markers of authenticity and quality for wine is still under study.⁴

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DETERMINATION OF ACTIVE SUBSTANCES BY HPLC-MS AFTER PROCESSING WASTE FROM WINE INDUSTRY USING VARIOUS EXTRACTION METHODS

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Wine waste formed during wine production can comprise valuable bioactive substances e.g., resveratrol and viniferin, important in food production and very useful in a various branches of science thanks to their potential antioxidant activity. To obtain the relevant amount of those stilbenes it is essential to use effective extraction method. Considering that, comparison of several different extraction methods (maceration, ultrasonic extraction, Soxhlet and pressured liquid extraction) under various conditions such as different solvents, extraction time, temperature and exposition to the light has been performed. Results were obtained by newly modified HPLC-MS method which is proved to be accurate, reproducible and efficient for determination resveratrol and viniferin. Depending on extraction time and used method, it was found that higher temperature leads to decomposition of stilbenes. The greatest concentrations found were 13120 µg/g d.w. of *trans*-resveratrol, 9460 µg/g d.w. for *cis*-viniferin and 4150 µg/g d.w. for *trans*-viniferin. The most noticeable results were obtained using maceration as an extraction method in dark with the following exposition to the light. After verification by the NMR, as a result of light exposure, dimerization between two molecules of *trans*-resveratrol was confirmed. Light-initiated isomerization of *trans*-stilbenes cannot be explained by this mechanism.

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ENVIRONMENTAL IMPLICATION BETWEEN CONVENTIONAL AND ORGANIC VINEYARD MANAGEMENT

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Sustainable practices are increasingly recognized by the wine sector as important innovations to reduce the environmental impact. The debate of applying organic viticulture, which reduces the impact on the environment, or conventional viticulture, which ensures an increase of yield, affects the decision to move towards sustainable solutions [1] Life Cycle Assessment (LCA). In this work, a comparison of environmental impact and the opportunity cost of applying conventional or organic viticulture was implemented. Environmental indicators (Water Footprint, direct Carbon Footprint, and Territory [2], [3] greenhouse gases emissions and the use of Life Cycle Assessment methodology. Among the environmental indicators the carbon and the water footprint are often used. These indicators, while being useful to assess the sustainability performance of the winegrowing farms, do not take into account important aspects related to the agronomic management of the vineyard. To fill this gap a new indicator called "Vigneto" (Vineyard in Italian language) and the economic aspect of sustainability such as the marginal benefit have been assessed to compare the two management in the vineyard. Finally, a sensitivity analysis has been applied to analyze the variability along the time-series. This study shows that organic management decreases the Water Footprint and Carbon Footprint thanks to lower amount of fertilizers applied and field passages. Territory indicator presents a better performance under organic management and the marginal benefit is greater thanks to the higher marketable price and a lower management cost. Organic management shows a better marginal benefit and a better environmental performance. The organic management in viticulture can preserve the natural capital without affecting the economic benefit and vice versa.

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IN A RAT MODEL OF AGING, LONG-TERM TREATMENT WITH WINE ANTIOXIDANT, RESVERATROL, UPREGULATES PLASMA LEVELS OF TESTOSTERONE IN MALES AND DOWNREGULATES PLASMA LEVELS OF PROGESTERONE IN FEMALES

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Aging-related impaired body structure and functions are assumed to be, at least partially, caused by elevated oxidative stress (OS). Resveratrol, a polyphenol in grapes and red wine, may act as an antioxidant and anti-aging compound, but its actions *in vivo* are controversial (1). Here we report on the long-term sex-related effects of resveratrol treatment (RT) on blood/plasma parameters of DNA damage, oxidative status, and sex hormone (SH) concentrations in a rat model of aging (2). Starting from their age of 3-mo, for the next 9-mo or 21-mo male and female Wistar rats were given resveratrol in water (10 mg/L; predicted intake: ~1 mg/kg b.m./day) or only water (controls). In their blood/plasma we performed the alkaline comet assay (3) and determined glutathione (4) and malondialdehyde (5) by established methods, whereas SHs were determined by commercial kits. Compared to controls, the 9-mo RT did not change any parameter in either sex. After the 21-mo RT, the only change noticed was the ~45% higher ($P < 0.001$) testosterone levels in males, and ~40% ($P < 0.01$) lower progesterone levels in females. The data indicate that the resveratrol-rich wines may benefit the health of aging men by stimulating their level of testosterone, while in aging women, the same wines may not be health-promoting.

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CEO'S ENTREPRENEURIAL PROFILE AND SURVIVAL OF INTERNATIONALIZED WINE SECTOR SME IN PORTUGUESE REGION OF RIBATEJO

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This study highlights the importance of the CEO's entrepreneurial profile, unveiling the preponderant role it assumes for the survival and internationalization of small and medium-sized enterprises (SME) in the wine sector in the Portuguese region of Ribatejo. Evidence shows that the CEO, as individuals of global mentality, are much more awake and available to continually seek international opportunities to ensure the achievement of additional benefits. Thus, they have to overcome different barriers with which they face in the course of the internationalization process, determining the decision-making mechanisms that involve different modes of entry into new markets, always bearing in mind the sources of competitive advantage, in order to ensure, in future terms, greater financial sustainability and responsible profit sharing. The empirical approach makes use of a qualitative methodology, based on interviews with the CEO of two wine companies located in the Portuguese region of Ribatejo. This study provides important implications for the strategic business process management aimed at overcoming obstacles to the internationalisation of wine-growing SME, through the choice of adequate entry modes.

Key-words CEO; Family Industries of Wine; Internationalization; SME.

LAMP- A FAST METHOD FOR ON-SITE DETECTION OF FUNGAL INFECTIONS IN GRAPES

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Loop-mediated isothermal amplification (LAMP) (Notomi, 2000) is a novel technology for the amplification of DNA sequences with high specificity, sensitivity, and rapidity under isothermal conditions. LAMP is a promising tool in a wide range of applications described in literature, *inter alia*, for the detection of *Botrytis* (*B.*) *cinerea* as the causal agent of *Botrytis* bunch rot. We developed such assays for the detection of *Penicillium* (*P.*) *oxalicum*, whose proteins are supposed to induce gushing in sparkling wine, and for the detection of patulin-producing *Penicillium* species. Different grape and soil samples from vineyards in Germany collected during the harvest 2018 were tested with LAMP assays for the specific detection of *P. oxalicum*, *B. cinerea*, and patulin-producing *Penicillium* species. The LAMP assay for the detection of patulin-producing *Penicillium* species revealed no positive sample. One grape sample was positive for *P. oxalicum*. Twelve grape and ten soil samples were positive for *B. cinerea*. The application of LAMP assays for the testing of grape and soil samples is very suitable for the detection of fungal infections. Its simple handling and in-tube detection of signals make this method advantageous and economically affordable for on-site investigations in the beverage industry. For easier application in the field ready-to-use-LAMP assays will be prepared in a vacuum-dried format.

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THE ROLE OF PAU5 IN GUSHING OF SPARKLING WINE

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Gushing is defined as spontaneous excessive over-foaming of carbonated beverages after pressure release by opening of a bottle despite correct handling (Schumacher, 2002) and causes severe ecological and reputational damages to the producers. In this study, the protein seripauperin 5 (PAU5) from *Saccharomyces cerevisiae* was identified as a biomarker for gushing with a direct gushing-reducing effect in sparkling wine. A practical concept for the reduction of gushing in sparkling wine based on PAU5 shall be developed. PAU5 was relatively quantified via RP-HPLC analysis. Purified PAU5 was added to grape juice before foam decay measurements. The gene was cloned into *Pichia pastoris* and *Saccharomyces cerevisiae* for heterologous protein expression. Low amounts of PAU5 occurred in gushing-positive sparkling wines and PAU5 had a stabilizing effect on foams indicating direct gushing-reducing effects. PAU5 is highly O-glycosylated and has structural similarities to yeast mannoproteins that are assumed to prevent gushing in sparkling wine (Bach, 2001). PAU5 expression in different yeasts delivers different glycosylation patterns and thus enables investigation of the impact of glycosylation on gushing-reduction. The yeast protein PAU5 is a biomarker for gushing in sparkling wine and enables development of gushing-reducing strategies.

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PORTUGUESE VINE CANE AS A SUSTAINABLE SOURCE OF BIOACTIVE COMPOUNDS

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Vine-canes are an important waste in all viticulture areas that should be re-used with innovative applications. Portuguese vine canes are a good source of polyphenolic compounds which have been associated with several health benefits [1]. The aim of the present study was to characterize the vine canes from six important Portuguese grape varieties from Douro, Dão and Minho regions for further use as a source of phenolic compounds. For this purpose, different environmental-friendly extraction techniques were employed, and the phenolic composition and antioxidant activities were quantified by spectrophotometric and gas (GC-FPD) and liquid (UHPLC-MS/MS) chromatographic techniques. The highest concentrated extracts were obtained by subcritical water extraction with Touriga Nacional variety from Douro region presenting the highest total phenolic content (35.2 ± 1.7 mgGAE/gdry sample) and the highest antioxidant activity. UHPLC-MS/MS analysis enabled the identification of phenolic compounds belonging to different families, with gallic and caffeic acids, catechin and epicatechin being the main contributors to the antioxidant activities of the extracts. The pesticide control performed enable to guarantee the safety of pruning wastes. The present results demonstrated the potential of Portuguese vine canes to be used as a source of phenolic compounds, for further application in cosmetic and/or pharmaceutical industries.

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MORE THAN JUST RESVERATROL. IDENTIFICATION OF THE MOLECULAR MECHANISM BEHIND THE NUTRACEUTICAL ACTIVITY OF WINE, GRAPES AND POMACE

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The consumption of grapes, pomace and wine is beneficial for the treatment of several metabolic diseases, including cardiovascular diseases and atherosclerosis. Previous investigations have shown that these physiological effects cannot be only ascribed to Resveratrol and that other phytochemicals present in the phytocomplexes must be taken in account. The identification of their mechanism of action is, however, not a simple task since these act in synergism and influence each other's chemistry, biology and pharmacology. In the experimental pipeline here presented, we make use of in vivo biological assays and untargeted metabolomic approaches to identify the molecular details of wine phytocomplexes activity (1). Our pipeline involves murine models and human volunteers and an analysis of biological samples from them obtained by high resolution mass spectrometry techniques DI-FT-ICR-MS, GC-TOF-MS and LC-MS-MS (2). This pipeline can be applied to any phytocomplexes and was successful in identifying the molecular mechanism behind the TMAO lowering activity of Taurisolo (3), a grape pomace obtained from Aglianico, a cultivar grape from South of Italy. The metabolomic analyses of biological samples obtained from animal and volunteers contribute to highlight the molecular details underpinning the nutraceutical potential of grapes, pomace and wine.

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WINE WASTE PROCESSING – EXTRACTION OF RESVERATROL AND VINIFERINE

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Like any other industry, the winemaking industry produces a significant amount of wastes which are commonly used as fertilizers or they are combusted [1], even though they still contain significant amount of biologically active substances. This work is focused on the extraction of grape cane, a wooden material containing stilbenes (resveratrol or viniferine) which exhibits strong antioxidant, anti-inflammatory or antibacterial properties [2, 3]. To obtain isolate rich in resveratrol and viniferine, maceration, Soxhlet extraction or ultrasound assisted extraction was used. The chemical composition of isolates was determined via HPLC/MS. Extracted material from the South Moravian region contained 0.2 – 9.6 wt. % of resveratrol and 0.1 – 9,5 wt. % of viniferine. The chemical composition of isolates varied based on extraction method and process conditions. Experiments maintained at lower temperatures were more selective for the extraction of viniferine, while using higher temperatures led predominantly to the resveratrol isolation. Adopting of ultrasound into the process of maceration had a significant effect on the decrease of extraction times from days in case of maceration to minutes when using ultrasound. Results obtained from laboratory experiments served for the construction of semi-production multipurpose extraction unit which allows combining mentioned extraction methods in one operational unit.

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MYSENSE: AN INTEGRATED SERVICE PLATFORM TO SUPPORT PRECISION VITICULTURE PRACTICES INCORPORATING ARTIFICIAL INTELLIGENCE

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Vitis vinifera L. grapevine varieties identification plays a fundamental role when managing wine demarcated territories, such as Douro Demarcated Region (DDR), where more than 115 varieties are used to produce wine products (1). Ampelography is a major challenge for (human) experts who, by observation alone, should be able to distinguish different varieties and/or other mutations resulting from the effects of climate changes, which, not infrequently, present a high degree of inter- and intra-set similarity. The mySense environment, developed at the University of Trás-os-Montes e Alto Douro (UTAD) as a support tool for viticulture, incorporates an artificial intelligence (IA) engine that has been tested by the Instituto dos Vinhos do Douro e Porto (IVDP), with the objective of achieving an effective tool to support both its regulatory and inspecting responsibilities (2). The mySense grapevine variety identification service (Figure 1) is based on the submission of up to 6 images framing the element to identify. These are then analyzed by a Distributed Computational Intelligence Module (DCIM) that provides a probable identification. DCIM is based on a convolutional neural network (CNN) - Xception architecture – initially trained using a data set acquired in DDR and periodically improved by incorporating new validated observations. Currently, the system already allows to correctly identify 6 DDR noble grapevine varieties and is now widening the knowledge base for a correct identification of a larger set of grapevine varieties. The same architecture also provides a simple, yet practical, mechanism to record grapevine growth stages, according to the Baggiolini scale, by variety and location based on a set of photos. Results obtained thus so far allow to conclude that AI techniques can indeed contribute to a both quick and precise grapevines variety identification, endowing viticulture stakeholders with straightforward technological tools that will help support their regular activities.

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TANNIN COMPOSITION OF MONASTRELL GRAPES AND WINES AFFECTED BY PRE-HARVEST ELICITORS TREATMENT AND COLD MACERATION

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Tannins play an important role in the organoleptic properties of wines, and their levels in both grapes and wines can be achieved by several means. One way is based on the use of elicitors, agrochemicals whose action mechanism has been found to increase polyphenol levels. On the other hand, due to the importance of tannins in wine, different winemaking techniques based in low temperatures (cold soak or must freezing) have been proposed to release phenolic compounds and to improve the extraction of pigments, proanthocyanidins and aromas from grape skins to wine [1]. Preharvest application of two elicitors: methyl jasmonate (MeJ) and benzothiadiazole (BTH) and a mixture of the previous one on Monastrell vines was carried out during two seasons. Tannins in grapes and wines were analyzed according to Gil-Muñoz et al [2]. The results showed different tannin concentrations depending on the season, obtaining higher concentrations in all treatments during the first one. All the treatments show higher results in grapes treated with all treatments during 2016 and in wines when the cold maceration was used. Elicitors can be a good strategy to increase the tannin content in Monastrell grapes and wines, and additional winemaking process also can increase the extractability of these compounds.

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FOOD POLYPHENOLS INTERACTION WITH BITTER TASTE RECEPTORS AND WITH SALIVARY PROTEINS

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Polyphenols occur in plant-based foodstuffs and contribute directly to their flavor, e.g. astringency and bitter taste [1]. These taste properties can be appreciated in some foodstuffs (e.g. beer, coffee, red wine) but generally they are unwanted and efforts are taken to reduce them. As a result, a knowledge on how/which polyphenols are bitter and/or astringent is important for a focused modulation of these sensory properties. It is well-known that bitterness is perceived by activation of bitter taste receptors (TAS2Rs) [2]. Still, the mechanism for astringency onset has been a debated topic. Several mechanisms have been proposed to explain its onset (e.g. activation of trigeminal ganglion [3], binding to oral pellicle [4]). A general agreement is that the precipitation of salivary proteins (SP) by food polyphenols occurs and contributes to the overall astringency. Herein, the bitterness of polyphenols from different classes (procyanidin dimers type B, anthocyanin, hydrolyzable tannins and phenolic acid ethyl esters) was studied by a cell-based assay [5]. The TAS2Rs activated by these polyphenols and the half-maximum effective concentration (EC50) of each agonist-TAS2Rs pair was determined. TAS2R5 seems to be the only receptor “specifically” activated by natural (condensed) tannins, while TAS2R7 seems more tuned for hydrolyzable (ellagi)tannins and an anthocyanin. Additionally, it was observed that the presence of SP could impair the activation of TAS2Rs by some polyphenols.

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

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The use of yeast protein extracts (YPE) as fining agents arises from the allergic properties observed in classic protein-based fining agents [1]. It is widely known that fining can remove soluble substances, including polymerized tannins and coloring matter in red wines affecting the organoleptic properties of wine [2]. As polyphenols composition affect wine organoleptic properties, the aim of this work is to understand the molecular mechanisms as how YPE-wine polyphenols interactions could modulate the color as well as the taste sensations after wine fining with YPE (developed by Proenol, Biotechnology Industry). Briefly, polyphenols related with astringency and bitterness were analyzed by LC-MS. The influence of YPE on wine color was also assayed by CieLab system. The effect of YPE-wine on the interaction with salivary proteins was also analyzed by SDS-PAGE after wine ingestion during a sensory evaluation. Overall, it was concluded that wines clarified with YPE revealed a significant decrease in the majority of identified compounds related to bitterness and astringency. The study of wine color revealed that YPE had the ability to reduce yellow color of white wines and did not remove red color of red and rosé wines, which is an important aspect in consumption market.

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Poster Communications

ANALYSIS OF WINES BY MEANS OF A IMPEDIMETRIC TONGUE USING LAYERED PEDOT:PSS NANOCOMPOSITES MODIFIED WITH NANOPARTICLES AND PHTLALOCYANINES

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An impedimetric electronic tongue to discriminate red wines as a function of its origin, vintage and ageing is developed. The multisensor system is formed by one PEDOT:PSS sensor and two nanocomposites formed by layers of PEDOT:PSS and gold nanoparticles (PEDOT:PSS/AuNP) or layers of PEDOT:PSS and lutetium bisphthalocyanine (PEDOT:PSS/LuPc₂). The smaller R_{ct} values revealed a low electron-transfer resistance which might be caused by the enhanced electrocatalytic effect caused by the interaction between PEDOT:PSS and the AuNPs and LuPc₂ deposited films. The improvement of the observed performance can be attributed to the enhanced electron transfer rate provided by the composites due to the interactions between two electrocatalytic components. This electrocatalytic activity of the sensing materials is promoted in the layered composites and enhances the cross-selectivity of the sensors. The good performance achieved is due to the remarkable sensing properties of the nanocomposites forming the array and to the methodology developed to extract the input variables used in the statistical analysis. A new feature extraction method based on an equivalent circuit model to obtain the input variables used in the statistical analysis has been developed. Using statistical procedure of Principal Component Analysis, the improved electronic tongue is able to discriminate three wines from the variety Tempranillo, one wine of the variety Tinta de Toro (a clon of Tempranillo) and one wine elaborated with a coupage of 90% Tempranillo and 10% Garnacha variety. The combination of layered nanomaterials and the new feature extraction method also enhances the capability of the impedimetric electronic tongue to predict chemical variables using Partial Least Squares regression.

MADEIRA WINE AGEING AROMAS: MONITORING THE *ESTUFAGEM* AND *CANTEIRO* PROCESSES

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Madeira wine (MW) production is distinguished from others by the way the ageing process is conducted. This can include *estufagem*, which consists of artificial heating the wine at about 45 °C for 3-4 months in stainless steel tanks. The wines with superior characteristics follow *canteiro* ageing, where the wine remains in wood casks, usually placed in the upper floors of the warehouses, exposed to temperatures between 15 to 30 °C, for a minimum period of 3 years. During both processes, oxidative ageing takes place, resulting in the formation of key aromas, such as sotolon and 5-hydroxymethylfurfural (HMF). In this study, both compounds were monitored (3-year study) during the *estufagem* and *canteiro* of dry and sweet MWs. The analyses were carried out by liquid chromatography [1, 2]. Both compounds significantly increase during the first year of ageing. The wines with the highest concentrations were those submitted to *estufagem* (29-405 µg/L for sotolon and 10-142 mg/L for HMF). *Estufagem* significantly accelerates their formation, up to 6-fold and 2-fold, respectively, mostly in sweet wines. Thus, *estufagem* is an efficient process for the enrichment of key aromas in sweet wines. New optical fibre sensor designs will be further explored for their real-time monitoring.

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EVOLUTION OF RED WINE IN CUSTOM OXYGENATION OAK WOOD BARREL. PHENOLIC COMPOUNDS AND COLOUR DURING AGEING

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The oak barrels aging improves wine chemical and sensory properties. The oxygen entry that occur during ageing in barrels plays an essential role, and its quantity depends on many factors, especially on species, anatomical characteristics, barrel type and cooperage treatment. The aim of this study was to evaluate the phenolic composition and color of the same red wine aged in barrels with different oxygen rates (OTR). The *Quercus petraea* oak staves were classified according to its OTR and barrels were built with high OTR (HOTR) and low OTR (LOTR), 4 of each one, in same cooperage. Red wine was aging in these barrels during 12 months and phenolic composition and color were evaluated. The wine ageing in barrels with different OTR were principally different at 6 months, probably due to the fact that in the first months of aging takes place the most oxygen input. At this time, LOTR wines presented more anthocyanins, Folin, PPP, PMP, %520 and less low molecular polyphenols, tannins and %420 than HOTR. It is possible to differentiate wine ageing in barrels with different OTR, the greater or lesser amount of oxygen that the wine receives affects its evolution in barrel, especially at 6 months.

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MICROBIAL COMMUNITY PROFILE OF ALVARINHO WINE GRAPES OF DIFFERENT GEOGRAPHIC AREAS

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According to recent metagenomic approaches to the determination of the fungal and bacterial composition of the grape associated microbiome, geographic origin is one determining factor of the fungal and bacterial consortia [1] and its influence in the organoleptic characteristics of fermenting musts may explain some of attributes related to terroir [2]. In the present work, the microbiome present on the surface of grapes obtained from ten Alvarinho vineyards in different geographic locations across Portugal is determined and analysed. Fungi and bacterial flora associated with the surface of wine grapes was removed by washing and sonication, and subsequently used to obtain purified DNA. Short-amplicon high-throughput sequencing of the fungal ITS region and bacterial 16S region was performed using the Illumina MiSeq platform. A total of 663 424 reads allowed the identification of 250 different bacterial species and 752 fungal species, being *Sphingomonadaceae* and *Sporidiobolaceae* respectively the bacterial and fungal families more predominant. Data provided insight into the microbial community profile associated with Alvarinho wine grapes. α -diversity analysis demonstrated that parcels from the Monção and Melgaço sub-region have a significant ($p < 0,05$) different diversity and species richness when compared to other regions.

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EVALUATION OF THE ANTI-HYPERGLYCAEMIC POTENTIAL OF WHITE WINE – INHIBITION OF INTESTINAL GLUCOSE TRANSPORT IN CACO-2 CELLS BY POLYPHENOLS

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Epidemiological and clinical studies have shown that a regular and moderate alcohol consumption is associated with reduced risk of developing type 2 diabetes [1]. In this field, red wine has been highlighted for its composition in phenolic compounds, including resveratrol and flavonoids, which present anti-hyperglycaemic properties [3]. For white wine, there are no studies exploring its anti-diabetic potential. Twelve wine samples produced in Portugal were characterized in terms of phenolic content by Folin Ciocalteu method and HPLC-UV-DAD/LC-MS. The effect of the gastrointestinal digestion on the phenolic composition was evaluated and the capacity of digested samples and standard compounds in inhibiting glucose transporters (GLUT2 and SGLT1) was assessed in a Caco-2 cell monolayers. Wine samples presented a total phenolic content ranging from 130 to 196 mg GAE/L and the major compounds identified included ferulic acid, p-coumaric acid and caffeic acid. Importantly, white wine efficiently inhibited glucose transporters in a dose dependent manner, and this effect was also observed for the digested fraction. Among wine phenolics, resveratrol and ϵ -viniferin significantly decreased glucose transport through Caco-2 cells whereas phenolic acids did not present significant inhibitory effects. White wine polyphenols showed to present promising effects on type 2 diabetes by inhibiting intestinal glucose transporters.

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INFLUENCE OF CELL WALL DECONSTRUCTING ENZYMES IN THE PROANTHOCYANIDIN-CELL WALL ABSORPTION/ DESORPTION PROCESS

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Only a small amount of the grape tannins effectively diffuses to must/wine. A large part of them are adsorbed to vegetal cell walls during vinification. The use of wall cell deconstructing enzymes could reduce these interactions. The objective of this study is to evaluate the capacity of 4 hydrolytic enzymes (added individually or together) to limit interactions between cell walls and tannins or to favor their desorption. Adsorption tests were carried out mixing a commercial seed tannin, purified cell walls from Syrah grapes and hydrolytic enzymes in model-wine solutions. In desorption test, enzymes were added after binding tannins and cell walls. Tannins remaining in solution were analyzed by HPLC (phloroglucinolysis reaction), SEC and the polysaccharides released from cell walls by SEC. Individual pure enzymes limit the interactions between tannins and cell walls, especially cellulase, pectinlyase and xylanase. On another hand, pectinmethylesterase, xylanase and the combination of the enzymes promote their desorption from cell walls and modified the composition of the liberated tannins. Some enzymatic activities favor the desorption of tannins from cell walls, prevent their interaction or have both effects. However, the excessive degradation of cell walls released a high amount of polysaccharides to the medium that also can bind tannins and this is an effect that needs to be considered.

ORIENTED ANTIBODY IMMOBILIZATION FOR ALLERGEN DETECTION USING SURFACE PLASMON RESONANCE SPECTROMETRY

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One of two major groups of methods for food allergen detection is immunological assay [1]. Among them surface plasmon resonance (SPR) biosensors are known for label-free and real time detection [2,3]. However, during immobilization of antibodies on the sensor surface their antigen-binding activity is usually lower in comparison with the activity in solution due to random orientation and steric hindrance. To prevent this, an intermediate layer of Staphylococcal protein A (SPA) known for selective binding of Fc-fragment of antibody, can be used. To ensure its reliable immobilization on the gold sensor surface, one cysteine residue was introduced into recombinant SPA (SPA-Cys) [4]. SPR spectrometry was used for the analysis of antigen-binding activity of immobilized antibodies. During antibody immobilization via SPA-Cys, the number of immobilized molecules was three times higher than that during their immobilization by physical adsorption. SPR sensor responses on injections of antigen (lactoferrin) solutions were on average 1.7 times higher for the case of oriented immobilization. Comparison of antibody immobilization by physical adsorption or via SPA-Cys on the sensor surface of SPR spectrometer, and their interactions with antigen demonstrated obvious advantages of oriented immobilization.

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AUGMENTED WINE GLASSES

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A number of recent studies have analyzed the influence of music and sound on food or drink evaluation (1). Crossmodal taste-sound congruence has been shown to have a perceptual impact on taste intensity and other qualities such as bitterness or sweetness (2). As multisensorial integration is more likely to occur in conditions of spatial and temporal proximity between the stimuli in different modalities (3), and music and taste are time-varying in nature, it seems interesting to develop technologies that favor these situations of synchronization and co-location, while allowing to keep track of multisensory interactive effects along time. Augmented glass. This device consists in a wine glass with sensors that are able to detect three main gestures of the user: when the cup is taken by the hand, airing the wine and when the liquid contacts the mouth while drinking. The sensing is performed through electrodes attached to the surface of the cup, next to the edge, and connected to capacitive sensors. A soundtrack is activated for each gesture, intended to emphasize sweetness, sourness or bitterness of the wine. We have presented these glasses in two public events, where users reported taste changes in the intended direction. Tasting wine while listening to different soundtracks may lead to different perceptions of flavours.

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ANALYSIS OF MUSTS AND WINES BY MEANS OF A BIOELECTRONIC TONGUE USING POLYPYRROLE/GOLD NANOPARTICLES AS THE ELECTRON MEDIATOR

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A voltammetric bioelectronic tongue (bioET) formed by sensors based on electrodeposited polypyrrole/Gold nanoparticles (Ppy/AuNPs) films combined with tyrosinase and glucose oxidase was built up and applied to the analysis and discrimination of musts and wines. Voltammetric responses of the array of sensors demonstrated the effectiveness of polymers as electron mediators and the existence of favorable synergistic effects between Ppy and the AuNPs. The strong electrocatalytic properties of the sensors and the selectivity induced by the enzymes provided intense and reproducible responses with an enhanced capability to detect compounds present in wines such as phenols and glucose^{1,2}. Using Principal Component Analysis and Parallel Factor Analysis it was possible to discriminate musts according to the °Brix and TPI (Total Polyphenol Index), and wines according to the alcoholic degree and TPI. Partial Least Squares provided good correlations between the bioET output and traditional chemical parameters. Moreover, Support Vector Machines permitted to predict the TPI and the alcoholic degree of wines, from data provided by the bioET in the corresponding grape musts. This result is of great interest for the food industry because it opens the possibility to predict some of the characteristics of the final wine, from the beginning of the vinification process.

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STRATEGIES FOR THE REMOVAL OF VOLATILE PHENOLS CAUSING SMOKE TAIN IN WINES

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Global warming favours an increase in forest disturbance, such as fires because of drought, wind or/and natural ignition sources (Overpeck et al., 1990). For winemaking industry, this is also a problem given that some molecules present in the smoke, may cause an off flavor described as smoke taint. In order to test different strategies for smoke taint removal, Cabernet Sauvignon and País wines were employed. Both wines were elaborated with grapes exposed to smoke from a nearby forest fire the week before harvest. Five different strategies based on the use of fining agents were employed: activated carbon (AC), AC with glucosidase enzyme (ACG), two polyaniline-based compounds, and a polymer for TCA removal were tested against a control without treatment. Each agent was applied following the manufacturer instructions. Volatile compounds determination and sensory analysis were carried out. Among the 77 volatile compounds determined, 8 were volatile phenols. The effects of each treatment were not dependent on the wine type. AC and ACG seem to be best strategies for volatile phenols removal but were also more aggressive in removing positive volatiles. The addition of fining agents based on activated carbon were the most effective strategies for smoke taint removal, but also resulted in the loss of positive aroma compounds from the treated wines.

FRACTIONATION AND CHEMICAL IDENTIFICATION OF MACROMOLECULAR COMPOUNDS IN RED WINE USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION - HPLC - AECD

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Wine tannins are well known for their astringent and bitter qualities, they are considered beneficial for human health due to their antioxidant properties. But, due to their high reactivity they can cause undesirable phenomena as turbidity. Therefore, one step to help solve this problem is to evaluate the conformation of the tannins in the wine solution and how to separate these polymers from other compounds to obtain a subsequent chemical analysis. Asymmetrical Flow field-flow fractionation coupled with multiple detectors were used for the fractionation and determination of the molar mass and the hydrodynamic radius of the red wines macromolecules. In addition, HPLC-DAD and AECD were used for the chemical identification of the fractionated molecules at 280 nm. The average molar mass of the wine aggregates are in a range of 45 kDa and are composed principally of anthocyanins glycosylated with glucose, and with other sugars such as galactose, rhamnose and mannose, which also were acylated with phenolic acids e.g., *p*-coumaric acid. The findings propose new tools and methodologies in the field of wine colloids, for future applications in terms of the potential importance and influence over different wine properties.

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MODULATION OF INFLAMMATION BY RED WINE EXTRACT THROUGH AN ACTION OF NLRP3 INFLAMMASOME PATHWAY IN MACROPHAGES

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Inflammation has been described as an initiator event of major diseases especially in the occurrence of cancers. In this study, we addressed a new effect of Red Wine Extract (RWE) in inflammation through a modulation of IL-1 β secretion and the NLRP3 inflammasome pathway. Using two different macrophages, one of which does not express the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), which is essential to form active inflammasome complexes that produce IL-1 β , we analysed the production of IL-1 β by ELISA and the expression of the key components of NLRP3 inflammasome. RWE decreases IL-1 β secretion and gene expression whatever line is used. Moreover, this strong reduction of pro-inflammatory IL-1 β is associated with a decrease of NLRP3 and ASC protein expression, which depends on the choice of inflammasome activator ATP or nigericin. For the first time, we highlight that a complex mixture of polyphenols, i.e. RWE, can affect the priming signal and the activating signal leading to inflammasome activation in macrophages and subsequently inflammation.

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ASSESSMENT OF AROMA COMPOUNDS FROM AGED WINES WITH AMERICAN AND FRENCH OAK CHIPS

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Ageing is crucial in the production of many alcoholic beverages and wood constituents have a decisive contribution to the aged character. Modern trends in winemaking attempt to speed-up chemical changes in wines using oak chips. This study investigated the volatile composition and sensory quality attributes of red wines aged for 1.5 and 3 months, with American and French oak chips. Fetească neagră (*V. vinifera*) red grapes were obtained from North-East, Romania winemaking region. The extraction of minor aroma was obtained according to *Tredoux et al.* (2008) and determination was realized by using the sorptive extraction and gas chromatography coupled with mass spectrometry (SBSE-GC-MS). Clear differences between American and French oak chips were observed. After 3 months of ageing, the concentration of cis-whiskey lactone and guaiacol were higher for American oak samples, similar to *Martínez-Gil et al.*, 2018. However, the concentrations of furfural, 5-methylfurfural, 4-vinylguaiacol and trans-whiskey lactone were higher in wines aged with French oak chips. This indicates that French oak chips produce prominent smoky, licorice and toasty aromas, whereas American oak chips generate wines with obvious vanilla, toasty and cacao aromas. The chemometric analysis confirms the evolution of aroma compounds and provides a quantitative basis for discriminating red wines age with American and French oak chips.

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AROMA PROFILE OF *PICHIA MANSHURICA*: AN EMERGING WINE SPOILAGE YEAST

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Pichia manshurica species is associated to spoiled wines from Italy and Patagonia. The spoilage capacity of 11 *P. manshurica* strains– isolated from spoiled bottled organic wines of Montepulciano d'Abruzzo was evaluated in must and wine through the determination of aroma compounds according to Tofalo et al. (2016). Moreover, for its prompt identification a specie specific primer pair was developed using 26S rRNA as target gene. More than 100 components were identified belonging to alcohols, aldehydes, ketones, esters, acids, phenols, and aromatic compounds. The differences were quantitative rather than qualitative and a higher production was observed in wine. The majority of the strains released compounds associated to off-flavors and off-odors in both conditions. Primers were tested on different yeast species and a band of 206 bp was obtained only for *P. manshurica* strains. PCR products were purified and sequenced to confirm the specificity. Obtained data revealed the ability of *P. manshurica* strains to release compounds which could produce off-flavors and odors in wines. For the first time a quick, reliable and inexpensive way to identify this spoilage species was reported.

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INFLUENCE OF GRAPES GEOGRAPHICAL ORIGIN AND YEAST STRAIN ON AROMA PROFILE OF VALPOLICELLA RED WINES

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Grape origin is a relevant factor for wine aroma. Interactions between different factors means that each site can produce grapes with a unique chemical fingerprint (1). We have previously reported differences in wine's volatile composition, highlighting contribution of grape geographical origin, fermentation practices or ageing (2) (3). This study aims to interpret the contribution of yeast strain and grape geographical origin to wine volatile composition and sensory characteristics. Experimental red wines were produced with Corvina and Corvinone grapes obtained from two different geographical areas within the Valpolicella region, fermented with four different commercial yeasts plus a spontaneous fermentation. Samples were analysed by gas-chromatography techniques and sensory analysis. Differentiation attributable to yeasts is observed, concerning esters content, but also δ -decanolactone and furfural. Differences due to geographical origin were mostly associated with vanillates and terpenes. For Corvinone wines β -damascenone also differentiated geographical origin, while for Corvina wines β -citronellol played a role. Sorting task showed that, in Corvinone wines, geographical origin had greater influence than yeast strain, whereas in Corvina the situation is more complex. This study shows that the influence of geographical origin is present despite the use of different commercial yeasts.

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WATER FOOTPRINT OF THE WINE CHAIN: COMPARISON BETWEEN TWO PORTUGUESE CASE STUDIES

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Water scarcity caused by climate change and its implications on grape production and quality have raised concerns among wine producers. The adoption of sustainable practices is now a goal of winemakers since the efficient use of resources allows them to reduce production costs. The WineWaterFootprint project evaluated the water footprint in the wine industry through the development of a methodology applied to two case studies, along two years of monitoring. The results show that the water footprint of the vine is the production phase with the greatest impact, representing more than 98% of the total value. In the case study I the green water footprint is the most relevant component while in the case study II is the blue water footprint, accounting for about 70% and 55% of the total value, respectively. Overall, the water footprint ranged from 370 to 610 L of water per bottle of wine produced (0.75 L) and is therefore similar to other studies reported in the Mediterranean region. The evaluation of the sustainability of the water footprint, through the analysis of life cycle, allowed also the identification of critical points. Water reuse is a way of reducing the impact of wine production on natural resources.

Key words: wine waste water, life cycle analysis, water use efficiency, irrigated vine

ISOLATION OF NON-SACCHAROMYCES IN TRADITIONAL WINE MADE FROM KALECIK KARASI TAKEN FROM ANKARA IN TURKEY

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Kalecik Karası is local red grapes in Ankara, Turkey. Traditional wine was made these grapes and yeasts were isolated at different maceration time in this wine. After isolated with specific agar, the yeasts were identified by sequencing with respect to ITS region. 5 types of non-*Saccharomyces* (*Hanseniaspora guilliermondii*, *Hanseniaspora opuntiae*, *Rhodotorula mucilaginosa*, *Hanseniaspora uvarum*, and *Wickerhamomyces anomalus*) were found in maceration time. Selected isolates were tested some properties such as alcohol tolerance, SO₂ tolerance and H₂S production. In addition, the volatile compounds of traditional wine and commercial wine made with these grapes were analyzed. According to the result of volatile compounds analysis, fruity (3-Ethoxy-1-propanol), rose (phenylethyl alcohol) and floral (phenylethyl acetate) aroma were enhanced much more in the traditional wine than the commercial wine. On the other hand, banana (isoamyl acetate) and pineapple (ethyl octanoate) aroma were found more in the commercial wine.

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INFLUENCE OF GEOGRAPHIC ORIGIN OF ALVARINHO GRAPES VARIETY ON THE SENSORY PROFILE OF THE WINES

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The sensory profile and chemical composition of a wine can be altered by both viticultural and oenological manipulations [1]. Grape and wine processing operations have been shown to influence sensory profiles and chemical composition of wines [2]. The present work compared the sensory profiles of wines produced from the Alvarinho grape variety obtained from eleven Alvarinho vineyards in different geographic locations across Portugal, including sub-regions of Vinhos Verdes-VV (Monção-Melgaço, Basto, Lima, Cávado) and other national regions (Alentejo, Dão, Trás-os-Montes). Alvarinho wines production followed the same vinification procedure. Sensory analysis of wines was performed by panel of expert assessors from the Laboratory of the Viticulture Commission of the Vinhos Verdes Region, following the certified procedure. Additionally, a panel of 6 oenologists, expert assessors, performed a quantitative descriptive analysis using a 5-points-structured scale. Transparency and colour attributes of wines presented significant differences ($p < 0.05$), when evaluating the effect of the region. Aroma and taste quality attributes exhibited major differences ($p < 0.05$) from the sample from Alentejo and minor differences among wines from VV-Monção-Melgaço, VV-Lima and Trás os Montes. Data provided insight into effect of region on the sensory profile of Alvarinho wines evidencing that some similarity in the sensory profiles within the same viticulture region.

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CHARACTERIZATION OF THE CORK POLYPHENOLIC FRACTION FROM DIFFERENT REGIONS OF THE IBERIAN PENINSULA ABLE TO MIGRATE TO WINE MODEL SOLUTIONS

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Cork oak (*Quercus suber* L.) is a tree of high economic and ecological importance in Portugal and south-western Iberian Peninsula (Costa and Oliveira, 2015). Cork industry is one of the most important industries in Portugal with Portugal being the world's largest cork producer, with 60% of the total cork tree area providing about 80% of the cork produced in the World. In this work, the phenolic composition of cork from different geographical locations was studied and a correlation between these compounds and the cork geographical origin was made. For that, the polyphenolic compounds present in eleven cork samples from different geographical regions of the Iberian Peninsula (six Spanish and five Portuguese regions) were extracted with wine model solutions during 45 days. Twenty compounds previously identified in cork using LC-MS were quantified by HPLC in the different extracts and the quantities and presence or absence of some have showed to be depend from the origin of cork used. These preliminary results showed that the southernmost regions of the Iberian Peninsula (Algarve and Cádiz) are those that stand out in all the properties measured.

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SPATIALLY EXPLICIT MODELLING OF VINEYARDS BIOCLIMATIC INDICES FOR CLIMATE CHANGE ASSESSMENT IMPACTS IN THE ALTO MINHO (LIMA AND MONÇÃO-MELGAÇO SUBREGIONS OF VINHOS VERDES WINE REGION, NW PORTUGAL)

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The potential influence of climate on the quantity and quality of wine production can be assessed through spatially explicit models of bioclimatic indexes. The current and potential climatic changes question the definition of terroirs, the vineyards phenology dynamics and wine typicity with social, economic, landscape and regional / local environmental impacts. The use of climatic data (CLIMATE-EU) in the ENSEMBLE model allowed the analysis of the climatic evolution between the present (1960-1990) and the scenarios RCP4.5 and RCP8.5 (IPCC) for two periods (2041-2070 and 2071-2100) in Alto Minho region (NW Portugal). These data and procedures allow analyse the comparative evolution the cartography of bioclimatic indices Winkler (IW), Huglin Heliothermal (IH) and Selianinov Hydrothermic (K). The results indicate, between present and defined scenarios, trends to: potential vegetative cycles changes of vineyards; expansion of suitable areas for vineyard mainly at altitude and areas with high/extreme temperatures in interior valleys; reduction of vineyards areas with the present optimal conditions; decrease seasonal low air humidity and increase soil moisture/plant water stress and vineyards irrigation needs. The analyses reveals the strategies needs and the urgency of production technologies for mitigation and adaptation to climate change, including the management of risks, quantity, value and production costs.

IMPACT OF HIGH VOLTAGE ELECTRICAL DISCHARGE PLASMA ON THE PHENOLIC AND CHROMATIC COMPOSITION OF RED WINE DURING BOTTLE STORAGE

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The non-thermal processing of foods is mainly focused on microbial inactivation, food safety and preservation, while maintaining the product quality. With this in mind, the non-thermal plasma (NTP) could be applied as alternative method for reduction of SO₂ addition in winemaking. The aim of this study was to evaluate the effect of NTP along antioxidant addition (SO₂ and glutathione) on phenolic and chromatic composition during 12 months of storage. Red wine samples with different antioxidants additions (SO₂ and glutathione) were subjected to NTP. Treated wines along with untreated sample were analyzed immediately after treatment and after 3, 6 and 12 months of storage. Total phenolics (TP), total anthocyanins (TA), total tannins (TT), and the chromatic characteristics were analyzed by spectrophotometry, while individual phenolic compounds by HPLC-DAD/FLD. The results demonstrated that TP, TA, TT, free anthocyanins and flavan-3-ols decreased, while chromatic characteristics increased during observed storage period. Also, better protective effect was obtained by addition of SO₂ than glutathione, since chemical composition of these samples were less negative affected. NTP affected the phenolic and chromatic composition of red wine during storage, wherein the changes in analyzed parameters were most pronounced after 12 months of storage.

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ISOLATION OF FERMENTATIVE YEASTS FROM ANTARCTIC CONTINENT TO IMPROVE PRODUCTION OF CHILEAN WINE

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The selection of yeast strains that are viable and ferment at low temperature has great appeal to obtain wines without browning. Therefore, the isolation and characterization of fermentative yeasts from Antarctic areas would be interesting. Six soil samples collected from Fildes Bay (62°11'S, 58°57'W) and 3 soil samples from King George Island (62° 9,743' S; 58° 27,978' W) were processed for yeast isolation. Soil was suspended in sterile water and inoculums were seed into YM agar plates (1% glucose and antibiotics). Plates were cultured at 4, 10 and 18 °C. Non-filamentous colonies were selected and transferred into fresh YM agar plates (1% glucose). Isolates were analyzed macro and microscopically for subsequent determination of in vitro fermentative activity using inoculation method in tubes with a pH variation indicator. From collected soil samples, 296 isolates were obtained. From the latter, 227 have been microscopically characterized and 125 of them are psychrophilic yeasts (4 – 10 °C). The fermentative activity assay shows that 5 of the 20 processed samples have fermentative activity. We conclude that it is possible to isolate soil psychrophilic yeasts with fermentative activity from the Antarctic for their potential use in wine production.

GIS-BASED CLUSTERING FOR CHARACTERIZATION AND IMPLANTATION OF VINEYARDS IN REGION OF TÁVORA-VAROSA

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In 1989, the Távora-Varosa region was awarded the Denomination of origin, the first national wine-growing region demarcated for the production of sparkling wines. The morphological characteristics allow to create fresh wines with good acidity. Thus, it was intended to characterize and map the region in terms of elevation, slope, aspect and vineyard implantation. For the delimitation, the specifications of IWV1, CAOP2 and RDD3 limits were used. The altitude data were obtained from ASTER GDEM4. Slope and aspect maps were produced and the region was subdivided into a polygon fishnet 30x30m. The vineyard areas were obtained from COS20155. A cluster analysis with integration of the descriptive analysis using ARCGIS (v10.5) and SPSS (v25). There was an average elevation of 664.03m, maximum of 1106m and minimum of 193m, average slope of 9.210 and maximum of 45.040, being approximately 40% of the area of vineyard located above 600m. We agrouped the region in 7 clusters according to the variables and produced a digital cartography to support the analysis results. This study designed of a tool to support decision-making for the implementation of vineyard areas depending on the characteristics for the final product.

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A BIOGEOCHEMICAL STRATEGY FOR WINE AUTHENTICITY

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Authenticity is one of the contemporary concerns of the wine sector. Wine authenticity is critical to guarantee company's prestige, wine market price and ward off competitors. Wine market value is mainly associated with *terroir* and grape variety, resulting in highly expensive wines, therefore a preferred target for fraudulent practices. Additionally, today's consumers are more demanding and trust the label information to assist them in the selection and appreciation of a specific wine. Thus, an authenticity system is mandatory to protect producers, retailers, and consumers. The current wine traceability system is not capable of efficiently controlling all the production wine chain, thus requiring urgent measures to develop a reliable tracking system that assures wine provenance. Alternative scientific-technological solutions have emerged throughout the years, giving new insights and promising perspectives into the wine sector. However, none of the developed technologies can guarantee a specific and unique wine *terroir* per se. Consequently, a multidisciplinary approach is required to effectively control the entire wine chain. A group of different methodologies are presented, showing their interaction as a means to support a framework of integrated knowledge to be used in the wine industry.

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ESTABLISHMENT OF A PROTOPLAST-BASED PROTOCOL FOR GRAPEVINE TRANSFORMATION

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Grapevine (*Vitis vinifera* L.), is one of the most economically important fruit crops in the world, and its systematic sequencing projects produced many gene data sets that require detailed analysis. Transient expression systems are well suited not only for large-scale genetic studies but also for the characterization of genes which are involved in many fundamental mechanisms influencing the growth, development and quality of the grape [1]. Transient transformation strategies have been developed involving Agrobacterium-mediated and direct gene transfer methods. These last ones requires permeation of protoplast membranes by polyethyleneglycol (PEG) - or electroporation-mediated protoplast transfection. The isolation and regeneration of viable protoplasts deriving from perennial woody plants, including grapevine, is challenging, and are being continuously improved, as culture and regeneration of *Vitis* spp protoplasts is hampered by the presence of antioxidants and phenolic compounds produced by the mechanical damage occurring during cell wall digestion. In this context, grapevine cane leaves of different varieties (red and white) were tested, in order to increase viability and regeneration capacity of grapevine protoplasts. In preliminary experiments, the isolation procedure (enzymes concentration, duration of digestion, and purification) was optimized and results will be present in terms of protoplast yield and viability.

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EFFECT OF THERMOSONICATION AND PHYSICOCHEMICAL PROPERTIES OF WINE ON CULTURABILITY, VIABILITY AND METABOLIC ACTIVITY OF *BRETTANOMYCES BRUXELLENSIS* YEAST IN RED WINES

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The aim of this research was to investigate short- and long-term effect of thermosonication and different physicochemical properties of red wine on culturability, viability and metabolic activity of *Brettanomyces bruxellensis* yeast. Thermosonication (constant frequency of 20 kHz, 12.7 mm sonication probe and 120 μ m amplitude) was conducted at 43°C during 1, 2 and 3 min, while wine variations included several levels of pH, alcohol and sugar. Although culturability was not confirmed in dry wines immediately after 3-min treatment, thermosonication did not result in complete inactivation of *B. bruxellensis* population. It only affected slight decrease in viability (max 20.5%), while majority of population entered into viable but not culturable (VBNC) state. During storage, culturability was completely recovered, with almost 100% live cells. Moreover, thermosonication treatment resulted in lower production of volatile phenols. Lower pH and higher alcohol content influenced lower concentrations of volatile phenols, while sugar addition resulted in opposite trend. This research provides first evidence of VBNC state of *B. bruxellensis* after thermosonication exposure. Despite *B. bruxellensis* was not significantly affected by applied thermosonication, this technique could be useful for prevention of *B. bruxellensis* spoilage in early stages of wine contamination.

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DETECTION OF MADEIRA WINE AGEING AROMAS: MEASURING THE ODOUR IMPACT OF SOTOLON

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Madeira Wine (MW) oxidative ageing takes place at up to about 30 - 45°C, resulting in the formation of its key odorants. Despite existing preliminary studies about the sotolon odour perception [1], its impact in MW is still not fully understood. In this work, the odour perception of sotolon in MW was assessed using ASTM E679. Sensory analysis was carried out with different 3-year-old wines (dry and sweet MW samples from two different producers), commercially available, by 6 panellists, previously selected from 22 MW non-expert individuals. Another set of 3-, 5- and 10-year-old commercial blends comprised by 89 samples from four MW producers were analysed by RP-HPLC-MS/MS [2] to evaluate its sotolon content and the corresponding odour impact, calculating the OAV. Sotolon odour perception was as low as 23 µg/L. OAVs were found to vary between 0.1 to 22, with 16% being higher than 10, namely in older Blends (≥ 5-year-old). This study demonstrated that sotolon is relevant for the aroma definition of MW blends. Consequently, it is justifiable the development of new sensing strategies for real-time monitoring of sotolon in MW. In a near future, optical fibre sensors will be designed and implemented for this purpose.

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PEPTIDE COATED MAGNETIC NANOPARTICLES AS AFFINITY PROBE FOR DETECTION OF HISTAMINE IN WINE

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Intake of wine containing high amounts of histamine may cause toxicity episodes, especially in sensitive individuals. Herein, we demonstrate that magnetic nanoparticles (MPs) coated with CDFEDFEDFE peptide are capable of probing histamine. MPs were prepared using sodium borohydride reduction of iron salt. The peptide was synthesized using F-moc approach and characterized by mass spectrometry, spectrophotometry and FT-IR. Water solution of histamine was used as the sample. Approach is based on the adsorption of histamine on the peptide-coated nanoparticles, histamine elution and subsequent ion exchange chromatography analysis. Synthesized peptide was detected by mass spectrometry at 1686 m/z. Comparative FTIR analysis showed peak loss at 1190 and 1725 cm⁻¹ in histamine-peptide complex, which confirms deprotonation of carboxyl groups of Glu and Asp residues. Possible role of Phe residue in binding via π - π interactions was shown in spectrophotometry by strong peak formation at 260 nm. High affinity of generated nanoparticles was indicated by the percentage of histamine recovery. Preliminary results show that the designed MPs display high potential for detection of histamine.

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UPGRADING OF WINEMAKING RESIDUES FOR THE FOOD SECTOR

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Grapes generate huge amounts of solid residues (pomace) which are still rich in bioactive compounds including fibre and phenolics with potential healthy properties. However, the fibre fraction is mainly insoluble which is not the best from either a nutritional, or a technological point of view. Furthermore, some of the phenolics are present in a form bound to the cell walls with consequent low bioavailability. The overall objective of the Italian project ReMarcForFood (Fondazione Cariplo, 2016-0740 grant) is the development of new concepts of use for winemaking by-products based on their biotechnological conversion into innovative value-added ingredients through application of enzymatic treatments and their use as substrate for the growth of high protein mushrooms. Part of the project is aimed to develop a low-cost and environmentally friendly enzymatic hydrolysis treatment to obtain a fibre powder with improved phenolic compounds release and technological properties for food use. Different commercial enzyme preparations, including products already used in the winemaking process, and different operational variables (use on dried or fresh grape skins, enzyme concentrations, hydrolysis and time temperature) have been investigated. Oenological enzymes gave better results together with application before drying, which should also be the best from an industrial and environmental point of view.

HOW HAS THE WINE SECTOR INCORPORATED THE PREMISES OF CIRCULAR ECONOMY?

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Much has been done in the wine production sector to promote sustainable development. Given its relevance in the economy and in society, it is fundamental to align its activities with the optimal use of resources and the regeneration and restoration of the natural system in agreement with the premises set by the Circular Economy (CE). The main interest of this work was, through a systematic literature review, to identify in the revised studies, how the wine sector has incorporated the premises of CE in its activities. Six topics define the concerns addressed: water; solid waste; energy; chemical use; land use; and ecosystems, in different parts of the chain: viticulture, winemaking and distribution. From the evaluation of 41 selected empirical studies, no clear definition regarding CE was found; however, the detailed analysis reveals aspects which incorporate CE practices such as the reduction of waste disposal by its treatment and recovery, the best use of resources at all stages of the process chain and the need to rethink and redesign current practices. The specific subjects identified were waste recovery, alternative energy sources such as biofuels production and water quality improvement. Land use, ecosystem impacts, and use of agrochemicals were not considered.

Keywords Wine, Circular Economy, Resources, Systematic Review, Sustainable Development.

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DETERMINING THE ABILITY OF SOME COMMERCIAL YEASTS FOR REDUCING THE ALCOHOL CONTENT OF RED WINES

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The grape phenolic maturation is associated with wines with more body, intense color and fruity aromas [1]. However, high temperatures during ripening induce a faster technological maturity, leading to grapes with high sugar accumulation at the moment of harvest [2] and to wines with high alcohol content [3]. One method to reduce the wines alcohol content consists of using low alcohol yield yeasts [4]. In this study, three different commercial yeasts have been compared to a control yeast, during a red wine vinification. The physico-chemical, chromatic and sensory characteristics of the finished wines have been determined. The three assayed yeasts achieved a significant decrease in alcohol content. Two of them (U42 and Andante) also maintained the chromatic and physico-chemical characteristics of the wines. Sensory differences were observed and when a triangular sensory analysis was conducted, Andante, that lead to a 10% reduction of the alcohol content, was the only one that did not differentiate from the control wine. The three commercial yeasts reduced the wine alcohol content. Two of them, U42 and Andante, did not modified the wine organoleptic or chromatic characteristics.

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EVALUATION OF MUST TURBIDITY AND YEAST ASSIMILABLE NITROGEN ON THE AROMATIC QUALITY OF VERDEJO WINES

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The yeast efficiency in nitrogen consumption is one important factor on the development of the alcoholic fermentation of wines. Deficiencies on the nitrogen sources during this process can lead to a sluggish or stuck fermentation and so to the undesirable organoleptic properties of the produced wines. In this work, the influence on the aromatic quality depending on the yeast assimilable nitrogen (YAN) and the must turbidity was evaluated in Verdejo wines. Musts with an initial YAN content around 150 mg/L and musts with nitrogen supplementation (250 mg/L) were clarified up to 150 and 50 NTUs. The kinetic fermentation, the basic chemical characteristics and the aromatic composition (fermentative aromas and thiols) were determined. Moreover, a sensory analysis was performed by a certified (ISO 17025) tasting panel. Results showed the importance of the turbidity and YAN content on the wine composition and sensory characteristics. Wines with lower turbidity showed higher content on the all fermentative aromas (esters, alcohols, acetates and fatty acids). Regarding thiols, benzyl mercaptan and 3-mercaptohexyl acetate also showed higher content in musts with low turbidity and high YAN level. Accordingly, wines with lower turbidity also presented a fresher sensory profile.

OXYGEN CONSUMPTION KINETICS OF RED WINES DURING AGEING IN CUSTOM OXYGENATION OAK WOOD BARREL

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The control of oxygen received by barrel wines is essential to ensure their correct maturation and defines the kinetics of oxygen consumption, determining the final properties of the aged wine. The evolution of the same red wine aged for one year in 8 French barrels (*Quercus petraea*) has been studied, 4 barrels with high and 4 barrels with low oxygenation of the wine has been studied. At different stages of ageing, the oxygen consumption capacity of wines, their spectrum, metal and majority volatile content have been analysed. Wines saturated with oxygen consume it following a curve defined by parameters according to each wine. Thus, it has been found that the time required to consume 50% of the oxygen available is significantly longer in wines in low oxygen transfer rate (OTR) barrels, which also showed a significantly greater loss of iron. In addition, spectral information indicates that these wines have higher colour intensity than those aged in high OTR barrels. However, once oxygen consumption has ended, the wines from high OTR are those with the highest chromatic intensity. These results show the importance of the oxygen that wines receive during barrel aging, and the effect it has on the kinetics of oxygen consumption and therefore on the life of the wine.

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CHARACTERIZATION OF ANTHOCYANINS IN WINES DURING AGING IN CUSTOM OXIGENATION OAK WOOD BARREL

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During the red wine aging in barrels, interactions between compounds of wine, compounds released from wood, and oxygen take place¹. These interactions are generally considered to be important in red wine perception and colour qualities. In this study, the oak wood was classified by its potential oxygen permeability thanks to algorithms based on the anatomical and structural properties of the staves. Artificial vision was used for the recognition of these characteristics in each stave and thus to classify sufficient wood for the manufacture of the barrels. Subsequently, 8 barrels were manufactured with high oxygen permeability wood and 8 with low permeability wood. The resulting barrels made from high permeability wood had oxygen ingress rates twice as high as those made from low permeability wood. At the same time, barrels were made from unclassified wood that were used as control, and presented OTRs between the two types. A red wine from the DO Ribera del Duero was aged in half of the barrels for one year. We studied the anthocyanins profile evolution in the wine during one year. To evaluate the concentration and evolution of anthocyanins released from oak wood, wines were analysed by HPLC/DAD-MS, according to Mateus et al. method². After 6 months, several new compounds were found, significant differences in main compounds were observed. These differences were more significant after 12 months. In addition, for the first time a malvidin-3-O-lactateglucoside derivative was identified by HPLC-DAD-MS.

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APPLICATION OF TILE-BASED ALGORITHM FOR EVALUATION OF GRAPE COMPOSITION

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Grape development is influenced by a range of factors, such as genetic variability, terroir and viticultural practices. These factors have a direct impact on grape compounds and are the reason for their diversity. This work aimed to apply chemometric tools to assess if external factors affect the main composition of grapes. Samples of Touriga Nacional variety from grapevines in Douro and Dão regions were collected in four developmental stages. These sites ensured the exposure of vines to different terroir. Samples were analyzed according to a metabolomic protocol based in liquid chromatography coupled to mass spectrometry [1]. After extraction using water/methanol/chloroform (20:40:40, v/v/v), chromatographic separation was achieved with a reversed phase C18 column in gradient mode. Mass spectra were acquired in both positive and negative ionization mode and processed using a dedicated tile-based algorithm. Regarding major compounds, results have shown small differences for grapes collected at similar developmental state. Significant differences were found for samples collected at different time points. Minority compounds can reflect other differences, but further investigation is needed through refinement of the algorithm. Chemometric tools based on tile-based algorithms are suitable for assessing differences in grapes according to terroir.

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DEVELOPMENT OF A DNA-QCM BIOSENSOR FOR DNA HYBRIDIZATION DETECTION

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The occurrence of fraud in the wine sector has been a major concern, challenging researchers to develop reliable technologies suitable of guaranteeing wine authenticity. Currently, DNA-based techniques are being applied for this purpose, mainly because DNA resists to winemaking processes, making possible the application of grapevine fingerprinting throughout the wine-chain. Nevertheless, most of these approaches are time consuming, expensive and require specialized personnel and specialized facilities. To surpass these limitations alternative methodologies are being developed, with biosensor platforms being among them. Our group developed a DNA-QCM biosensor which combines the ability of single-stranded DNA to bind to its complementary strand, with a quartz-crystal microbalance capable of detecting mass changes in a scale of $\text{ng}\times\text{cm}^{-2}\times\text{Hz}^{-1}$. The presence of double-stranded DNA in the QCM surface leads to a mass change, which allows to detect hybridization between a DNA probe and a grapevine specific DNA target, both designed by our group. Our biosensor detected DNA hybridization between our probe and the grapevine DNA complementary target, being able to differentiate between the complementary target and a non-complementary sequence, differing only in three nucleotides. These results reveal the potential of this Biosensor platform for wine authenticity assessment, since this system is highly specific and cost-less.

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A MULTIDISCIPLINARY APPROACH FOR ALVARINHO WINE AUTHENTICITY

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The assessment of wine authenticity, through a varietal and denomination of origin certification, has become an increasing priority in the current context of a growing market globalization. Grapevine genetic background plays an important role in wine authenticity, especially when PDO wines are produced by a single variety (monovarietal wines). Single Nucleotide Polymorphisms (SNPs) rely on the identification of a specific locus in the DNA sequence, which is unique to a grapevine variety, allowing the use of high throughput detection platforms. DNA-based methods are not suitable for geographical determination, thus multi-isotope-ratio analysis has been used in wines signature, imposing their determination in the soil fraction so they can be extrapolated to wines. This study proposes an integrated authenticity system combining: 1) high-resolution melting (HRM) analysis for varietal identification, based on three genes belonging to the anthocyanin pathway [1,2]; and 2) the isotope ratios of wines and vineyard soils and rocks to trace the wine geographical provenience [3]. All Alvarinho SNP-HRM melting profile analysis, revealed the existence of a single haplotype/variant. The multi-isotope-ratios from Alvarinho wines and rocks from the three vineyards, from different appellations, proved to be a reliable approach to an accurate wine signature. The $^{87}\text{Sr}/^{86}\text{Sr}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ isotopic ratios are strongly correlated within wines and rocks from the same origin.

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IMPACT OF MECHANICAL AND MANUAL GRAPE HARVESTING ON PHYSICOCHEMICAL ASPECTS OF MERLOT MUSTS AND WINES PRODUCED IN THE CAMPANHA GAUCHA REGION - BRAZIL

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The Campanha Gaucha region shows a remarkable potential for the production of wines. There, the first grape mechanical harvester in Brazil were introduced. The objective of this study was to evaluate possible differences in the physicochemical characteristics of musts and wines elaborated with grapes obtained by manual and mechanical harvesting. The experiment was conducted in 2018, with two treatments (T1 - manual harvest, T2 - mechanical harvest), using 'Merlot' grapes. The parameters analyzed were density, reducing sugars, pH, total acidity, and tartaric and malic acids. Moreover, soluble solids, gluconic acid, ammonia, and potassium were analyzed just in musts, while ethanol, glycerol, lactic acid, Folin-Ciocalteu index, color intensity, and hue were analyzed just in wines. T2 musts presented higher values for most of the variables, and non-significant differences for density, total acidity, tartaric acid, and potassium. For wines, all the significantly different values were higher in T2, except density, and were respectively the following for T1 and T2: ethanol, 11.3 and 12.4%; pH, 3.68 and 3.73; density, 0.9934 and 0.9926; and Folin-Ciocalteu index, 46.08 and 50.27. In summary, the two different harvesting techniques influenced the physicochemical characteristics of musts and wines.

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ANTIBACTERIAL ACTIVITY OF GRAPE STEM EXTRACTS FROM SOUSÃO VARIETY: INFLUENCE OF CHITOSAN TREATMENT

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Antibiotic resistance bacteria are emerging and spreading globally threatening our ability to treat common infectious diseases¹. Therefore, the investigation and development of new antimicrobial agents are mandatory². Thus, we aimed to extract the polyphenols from the grape stems, treated with chitosan, and evaluate their antimicrobial activity against antibiotic resistant bacteria. Grapevines of the Sousão variety were sprayed (leaves and berries) with chitosan solution (concentration of 0.1%) and chitosan nanoparticles (concentration of 0.001%) at the beginning of veraison. Grape stems (treated and not treated with chitosan) were freeze-dried and mill-powdered. The extraction of polyphenols was performed with water/ethanol (50:50, v/v) mixture. Antimicrobial susceptibility was tested against 10 different bacterial species. Minimum inhibitory concentrations (MICs) were determined by agar dilution assay using different concentrations of polyphenol extracts. The stems extracts showed antimicrobial activity against all Gram-positive bacteria, except for *E. faecium*. The stems extracts from grapes treated with chitosan showed better results than the extracts from grapes treated with chitosan nanoparticles and the control group. The stems extracts were only effective against one Gram-negative bacteria. Stems extracts presented a higher efficacy against *Listeria monocytogenes*. Stems extracts present a high efficacy against Gram-positive bacteria, in particular extract from grapes treated with chitosan. From the Gram-negative bacteria tested only *Pseudomonas aeruginosa* showed susceptibility to extracts.

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DYNAMIC LIGHT SCATTERING - A PROMISING TOOL FOR BENTONITE CHARACTERIZATION

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The appearance of turbidity in white and rosé wine is a serious visual fault in winemaking, lowering the commercial value. To prevent haze formation, proteins are removed before bottling through adsorption onto bentonite. However, bentonite may adversely affect wine quality by inducing aroma losses and colour alterations. Therefore, it is pertinent to use the right quantity of bentonite. The total surface and the positive surface charge of bentonites correlates with the capacity to adsorb the negatively charged wine proteins. The aim of this project was to characterize commercial bentonites by Dynamic Light Scattering for size and surface charge. Dynamic Light Scattering was used to measure zeta potential for a characterization of the size and surface charge of commercial bentonites. The 13 bentonites studied show a different zeta potential and therefore a different capacity to bind proteins. Currently the efficacy of the bentonite to precipitate proteins and to prove the correlation between the zeta potential is under study. Zeta potential measurement is a helpful tool to characterize bentonites for their capacity to bind haze-forming proteins from wines. Due to the complex wines matrices, more analyses are needed for a final method validation.

MOLECULAR MODELING DESIGN OF HISTAMINE-BINDING PEPTIDES FOR ISOLATION AND DETECTION OF HISTAMINE IN WINE

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The detection of histamine is important for monitoring spoiled food or drinks particularly wine¹. Nanotechnology application of peptide-coated magnetic particles is very cheap and feasible for isolation of biomaterials². Sixteen protein crystal structures complexed with histamine (ligand id: HSM) in the Protein Data Bank were studied. We identified common amino acids binding the histamine amine tail terminus (Asp/Ser), the aliphatic tail (Phe/Tyr/Leu/Val) and the basic aromatic ring (Glu). Eight helical peptides were designed according to the following formula: Cys + [(Asp/Ser) + (Phe/Tyr/Leu/Val) + Glu]⁴. The Cys residue was added to allow binding of peptides to magnetic particles. Models were constructed using PEPFOLD3 server, whereas molecular docking GOLD program was used to evaluate histamine binding³. Molecular docking best fitness goldscores (ranked from 1000 dockings) were in the range 24.50-27.73 for all peptides. The CDYEDYEDYEDYE peptide showed the best results; contributed mostly by van-der-Waals (16.38) and H-bond (6.31) scores. Preliminary molecular docking showed that the designed eight histamine-binding peptides display similar fitness. Further molecular dynamics studies and wet lab validation is required to optimize specificity and selectivity for nanotechnological application in histamine isolation and detection.

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