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## EXTRACTION AND RETENTION OF TANNINS IN RED HYBRID WINE PRODUCTION

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Key words: Red hybrid wines, phenolic extraction, enological tannins, tannin sorption

Though essential to wine quality, the extraction and retention of grape-derived condensed tannins during red wine production is poorly understood, especially in interspecific cultivars. Tannin concentrations measured in source fruit correlates poorly with final wines<sup>1</sup>, with tannin range in grapes showing a 3-4 fold variation, but wines evincing a 50-fold range in tannin concentration (30-1895 mg/L)<sup>2</sup>. To explore factors influencing the tannin extraction in common red wine processing methods and retention post-fermentation, a series of hybrid grape fermentations were performed. In 2011, the phenolic profiles of must and wine from Maréchal Foch, Corot noir, and Marquette were characterized via solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) to assess the impact of cold soak, hot press, and enzyme or tannin addition during vinification. Treatments that increased skin contact or cellular degradation produced higher tannin concentrations in must, but carryover into finished wines was incomplete, suggesting that cultivar-specific factors may dictate final tannin concentration. This theory was tested in 2012, when the maximum recommended concentration of a grape-derived commercial tannin was added at various processing points during vinification of Maréchal Foch and Corot noir, and wine phenolic profiles were examined via SPE and HPLC. While all wines were found to be low in overall tannin, additions made later in wine processing, when fewer grape solids were present in the wine, favored higher final tannin concentrations. Later additions also favored greater mean degree of polymerization (mDP), though mDP averages for all cultivars were still relatively low (<6.0). These results correspond to earlier work suggesting that tannin addition rates developed for *V. vinifera* may be too low to effect sensory differences in hybrid wines, and that tannin sorption by grape solids varies by cultivar for both hybrid and *V. vinifera* grapes.

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# CLARIFYING THE MECHANISM OF PROTEIN HAZE FORMATION IN WHITE WINES

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Key words: wine, protein, haze, mechanism

Securing wine stability is an essential step of the winemaking process. Wine grapes contain proteins that, when not removed during winemaking, can make their way into the bottled wines in a soluble state, in which case the wine is seen as clear. With time, and particularly after exposure to high temperatures, the proteins can slowly denature and subsequently aggregate. As these aggregates become larger they scatter more light and they are seen as haze. Haze from wine contains mainly chitinases, thaumatin-like proteins and non-proteinaceous compounds, and is the result of a complex interplay between a range of proteins and chemical and physical factors. Since hazy wines are not saleable, this instability is generally prevented via bentonite fining, a treatment effective in removing the grape proteins responsible for haze formation, but with drawbacks such as wine volume loss and disposal costs, as well as perceived effects on wine flavor and quality. Hence, alternatives are sought. So far, the search for alternatives to bentonite has not yet resulted in commercially viable solutions able to compete with bentonite's efficacy and low cost. It is believed that a better comprehension of the causes for haze is needed, because a thorough understanding of the mechanisms of protein haze formation has the potential to lead to the development of novel, efficient, and environmentally sustainable winemaking processes to prevent haze from forming. Here we present an overview of the recent advances on the study of protein instability of white wines, with particular attention to those obtained by our group. This will include: i) notions on the role of purified wine proteins, ii) explanation of the role of some non-proteinaceous wine components, iii) notions on the effect of different unfolding temperatures and unfolding/aggregation behaviour of grape proteins. A revisited mechanism of haze formation accounting for the several recent breakthroughs in the field will be presented.

# RESEARCH OF NEW SWEET TRITERPENOIC DERIVATIVES IN OAK WOOD USING TARGETED SCREENING BY HIGH RESOLUTION MASS SPECTROMETRY

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Keywords: Sweetness, oak wood, Quercotriterpenosides, HRMS.

The research of key-compounds involved in wine aromas and tastes constitutes a major field of investigation in enology. Although sweetness is a subtle but crucial feature of dry wines quality, its molecular origin has been poorly studied. This lack of knowledge might partly originate from the high chemical complexity of wine. Therefore, the study of taste-active compounds appears as a great challenge for the understanding of wine flavors and requires the implementation of efficient and sensitive analytical techniques. Within this context, High Resolution Mass Spectrometry (HRMS) has recently seen impressive developments based on a metabolomic-inspired approach [1,2].

The present study reports a complementary use of Orbitrap-HRMS aimed at identifying isomers and derivatives of the triterpenic molecules Quercotriterpenoids (QTT) I and II recently discovered in oak wood and established as sweeteners [3]. An oak extract was screened by high accuracy molecular mass measurement in order to research targeted empirical formulas corresponding to putative structural analogues to QTT I and II. These researched compounds were observed in our extracts. So far, these derivatives of QTT have never been identified in nature. Their purification was performed by combination of centrifugal partition chromatography and high performance liquid chromatography guided both by HRMS. Thereafter, their structures were confirmed by NMR and their sensory properties assessed.

This study highlights the first identification of several triterpenic compounds, some of them exhibiting sweet properties, and improves our chemical knowledge of oak wood and wine composition. Furthermore the approach described in this work arises as a promising methodology to discover new taste-active compounds.

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## USE OF ENOLOGICAL ENZYMES TO CLARIFY GRAPE JUICE : EVIDENCE OF A CONTAMINATING PROTEASE ACTIVITY

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**Keywords :** Proteins, pectins, flocculation, pectinases, proteases, foam.

Clarifying enzymes containing pectinases are often used to clarify grape juice. The aim of such a treatment is to facilitate the wine filtration. Nevertheless, these enological products do not contain exclusively purified enzymes but all of the proteins released by *Aspergillus niger* during the fungus culture, including proteases. Now, the contribution of proteins in wine foaming properties is known for 20 years. Trials were performed with model grape berry juices to assess whether a “pectinases” treatment could alter the grape juice protein composition and its foaming properties. The complete protocol consisted of 8 trials, i.e all of the combinations with BSA at 200 mg/L, clarifying enzymes (Enz) and apple pectins (P) used at 200, 400 and 800 mg/L. All measures were made in triplicate 1, 3, 6 and 13 days after the treatment. Using a SDS-PAGE + CBB staining, it is observed that the protein quickly and regularly disappeared for the BSA+P trial without protein degradation. This is exclusively due to the flocculation mechanisms. After 6 days, protein content decreased by 90% for BSA+Enz and pretty completely disappeared for BSA+Enz+P. In the first case, BSA disappearance is explained by the contaminating protease activity present in the enological product. In the second case, BSA content decrease is due to the simultaneous action of pectinases and proteases. It seems that hydrolysed pectins were less capable to flocculate with BSA. For these 3 trials, severe decrease in foaming properties were observed when compared with the BSA medium.

Enological enzymes were also used to clarify a Chardonnay grape juice that was the 4<sup>th</sup> press cycle. The treatment induced a decrease in foaming properties by 20% as well as very low changes for the SDS-PAGE profiles when the control and the enzymes treated grape juices are compared.

Session de la conférence : SESSION I – Molécules actives du vin et procédés oenologiques  
Préférence: présentation orale

## **OXYGEN AND ELLAGITANNIN EVOLUTION AND ORGANOLEPTIC PROPERTIES OF FRENCH RED WINE AGED IN NEAR INFRA-RED CLASSIFICATION WOOD BARRELS**

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### **Keywords**

Oxygen, Ellagitannins, Red wine, Oak wood, Organoleptic properties, Astringency, Bitterness

### **Abstract**

During wine aging in oak barrels, ellagitannins such as vescalagin, castalagin, roburins A, B, C, D, E and grandinin are solubilized. These molecules concentrations are highly variable and depend of the oak wood species and origin as well as its treatment during barrel realization (seasoning, toasting). However, their kinetic of extraction and organoleptic impacts on red wine are still unclear [1].

The oak staves were classified in three different index of polyphénols (IP) groups (Low potential, Medium potential 1, Medium potential 2) by a NIRS online procedure (Oakscan®) [2] and three grain groups (extra fine (EFG), fine (FG) and medium (MG)) and assembled in order to make five different barrels (FG-LP, FG-MP1, FG-MP2, FEG-MP1, MG-MP1). Then, the barrels were equipped with small windows to measure the oxygen in wine with luminescence technology (PreSens®). In these barrels, a wine was aged and the concentrations of ellagitannins were followed by quantification of ellagic acid released during acidic hydrolysis of ellagitannins and by HPLC-UV-MS [1,3]. During this aging process, the organoleptic properties of wine were also estimated by a trained judge's panel at 6 and 12 months.

The oxygen concentration decrease quickly during the eight first days of aging. Moreover, these decreasing were significantly more important when the barrel IP and grain increase. The ellagitannins concentrations were also correlated with wood classification. During the first months of aging, an increase of their concentration was observed. Indeed, this increasing concentration in wine aged in barrel manufactured with the wood richer in ellagitannins was higher. Moreover correlation between ellagitannins concentration and oxygen consumption were observed. Furthermore, the organoleptic properties were significantly impacted since the wine with the smallest ellagitannins level was described with a lower roundness, astringency and bitterness.

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within a grape variety. Sensory analysis also proved that yeasts species alter the attribute intensities of wine within a single grape variety.

Topic of the conference: Wine Active Compounds 2014  
Session: Process-related active compounds  
Preference: Poster presentation

## **YEAST CO-INOCULATION AND RELEASE OF PROTEINS: A PROTEOMIC APPROACH**

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**Key words:**

non-*Saccharomyces* yeasts, co-inoculation, microbial interactions, proteins

Proteins secreted by yeasts during and shortly after alcoholic fermentation contribute to wine composition. The impact of extracellular enzymes in particular has been largely documented but the entire proteome has also been shown to relate the history of the process that led to the generation of a specific wine. The contribution of non-*Saccharomyces* yeasts towards some aspects of wine quality has been explored but although their ability to secrete enzymes of oenological interest has been reported, little is known about their global contribution to the wine proteome and if proteins can be regarded as markers of interactions with *Saccharomyces* sp., especially in the context of co-inoculation.

In this study, the proteins of different non-*Saccharomyces* yeasts released under fermentative conditions either on their own or co-inoculated with *S. cerevisiae* were identified. The results revealed that the number of proteins varies greatly between species. In mixed culture fermentation, proteins from both *Saccharomyces cerevisiae* and the non-*Saccharomyces* yeasts present were identified but although cell wall proteins could expectedly be identified in all wines, others were either only present in some of the wines or completely absent.

Proteins of obvious oenological interest identified in this study include various  $\beta$ -glucosidases, endochitinase and invertase. All of these have been reported in literature to have potential implications on wine quality. The identity of several proteins also suggests that specific interactions occurred between *S. cerevisiae* and the non-*Saccharomyces* yeasts. The nature of these interactions was dependent on the non-*Saccharomyces* species.

The untargeted approach used in this study to characterize proteins released by yeasts during wine fermentation broadened our knowledge and understanding of selected non-*Saccharomyces* yeasts, their interactions with *S. cerevisiae* and their potential impact on wine properties.



## STATISTICAL ANALYSIS OF METAL CONTENT OF WINES OF HUNGARY

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Key words: Tokaj-wines trace elements statistical methods

On the basis of the experience gained during the course of the recent years within the European Union and in Hungary as a part of it, in order to strengthen the reliability of the protection of origin of wine currently operating, it is obviously expected by the customers that the results achieved via analytical identification be announced.

Wine is considered as a highly complex balance system involving thousands of components which is objected to constant alteration until the moment of its consumption. Its quality seems to be influenced by four factors, or rather group of factors.

The physical and chemical compositions of growing areas appears to have an exceptionally crucial significance in the formation of the wine's features. Beyond the production of these sorts of wines, not only organoleptic but also analytical identification has a great importance. Browsing through technical literature one may provide the readers with several references to the applicability of various methods investigation or certain compounds for the identification of origin, a system capable of solving the problem with the necessary level of reliability at a reasonable price, on the other hand, has not been discovered yet.

One of the potential solutions is the determination of the fingerprint of wine samples. Our experiments have been conducted in the case of Tokaj-wines produced in different fields, processed in the similar ways. The results of the wines' organoleptic qualification and those of main and trace element analysis have been evaluated by the application of statistical methods.

**EVALUATION OF THE IMPACT OF A FUNGAL ORIGIN CHITOSAN PREPARATION ON *BRETTANOMYCES* IN THE CONTEXT OF WINE AGING**

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**Keywords :** *Brettanomyces*, chitosan, aging

*Brettanomyces bruxellensis* and the consequences of its development in wines are a continuous threat for wine quality. This undesirable yeast is able to develop during aging under difficult conditions. It is also responsible for the production of negative aromatic compounds such as volatile phenols related to sensory descriptors such as animal-like, horse, barnyard, band aid and medicinal. Nowadays at an international scale, volumes concerned by this defect become significant. Several strategies are useful in order to control *Brettanomyces* and its development in musts and wines. However these strategies are not always sufficient. In this context, chitosan of fungal origin has been introduced as a new potential interesting tool to control *Brettanomyces* in the context of winemaking [1]. Recent studies have showed the impact of a fungal origin chitosan application on wines contaminated with *Brettanomyces*, leading to the elimination of *Brettanomyces* cells, even at high levels of populations up to  $10^5 - 10^6$  CFU/mL and at the dosage of 4 g/hL [2]. In these studies, the chitosan preparation was added and the wine racked off after 10 days and the efficiency of the treatment was evaluated in a short delay after the treatment. Due to the necessity to control wine microbiological stability during the period of aging, especially in the context of red wine aging in barrels, our research focuses on the application of an enological chitosan preparation in order to prevent wine from *Brettanomyces* contamination along the aging period at both pilot winery- and at winery-scale. This communication presents the evaluation of the impact of different addition protocols of an enological chitosan preparation on *Brettanomyces* population evolution and volatile phenols content along the aging, up to 9 months. To conclude, the results confirm the interest of chitosan as a preventive tool to control *Brettanomyces* in the context of wine aging.

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# IMPACT OF ACIDITY AND OXYGEN CONCENTRATIONS ON CHEMICAL EVOLUTION AND PIGMENT FORMATION IN MERLOT RED WINE

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Keywords : Oxygenation ; Acidity ; Red Wine ; Pyranoanthocyanins ;

Microoxygenation is a widespread technique in winemaking. Indeed, it is known that oxygen can impact wine positively by decreasing herbal and reductive notes, as well as induce colour stabilization [1]. Chemically, the dissolved oxygen is able to produce ethanal by oxidizing the ethanol. Then, this ethanal can make ethyl bridges between flavonoids such as tannins and anthocyanins which can lead to huge polymeric structures [2]. The ethanal is also responsible for generating new pigments, called pyranoanthocyanins (vitisin B). These compounds result of a cycloaddition reaction between a vinyl group and the 5-OH group and C-4 of the anthocyanin followed by an oxidation step leading to the aromatization of pyranic ring D [3]. The aim of this work was to determine the impact of both oxygen and pH levels on the chemical composition and organoleptic properties on a young Merlot wine during 4 months.

The red wine pH was adjusted at 3, 3.5 and 4 with sulphuric acid and sodium hydroxide. For each pH, four quantities of pure oxygen were studied : 0, 2, 8 and 20 mg/L. Each week, oxygen was added according to wine consumption in order to keep the chosen amounts at the same levels.

First, global chemical impacts of both oxygen and pH were studied over a period of four months. Samples were analyzed each month in order to perform chemical follow-up of the wine. Free anthocyanins amounts showed a decrease related to oxygen as well as acidity. The same impact was observed on the evolution of free and total SO<sub>2</sub>. Colorimetric analyses showed an increase in color intensity according to oxygen amounts. However, the trend of evolution was clearer at acidic pH.

Secondly, after the period of four months, a pyranomalvidin-3-O-glucoside-procyanidin dimer was quantified in all the modalities. The results showed that, for the same pH, its quantity was correlated with oxygen concentrations. Moreover, for the same quantity of oxygen, the concentration of this pigment was related to acidity.

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## APPROACHING CARBON DIOXIDE DIFFUSION IN CHAMPAGNE WINES THROUGH MOLECULAR DYNAMICS

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Keywords : CO<sub>2</sub>, diffusion, champagne, molecular dynamics.

The physical chemistry of sparkling beverages, such as champagne, is a field of research that has been well advanced experimentally in recent years [1]. Indeed, several phenomena, such as diffusion, have been extensively studied [2]. Accompanying theoretical investigations which could further elucidate the experimental findings, are nevertheless rare, and hence the role of each type of molecule in the diffusion process remains unclear although diffusion is considered the main physical process responsible for the nucleation and growth of carbon dioxide bubbles in sparkling beverages.

In the present work we report a theoretical study of the CO<sub>2</sub> diffusion coefficient in a liquid, with a composition close to that of champagne. We have performed molecular dynamics simulations (integrating Newton's equations of motion) of CO<sub>2</sub> molecules in water and in a water/ethanol mixture respecting Champagne wine proportions. It is shown that, for champagne, generalized Fick's law for the determination of multicomponent diffusion coefficients can be approximated by standard Fick's law valid for binary systems. Diffusion coefficients computed from the Stokes-Einstein formula are in very good agreement with theoretical and experimental data from the literature. The addition of ethanol to a CO<sub>2</sub>/water mixture is found to be the main factor responsible for the value of the CO<sub>2</sub> diffusion coefficient in champagne, despite the fact that these sparkling beverages are multicomponent drinks. This implies that the CO<sub>2</sub> diffusion coefficient in Champagne wines should not depart significantly from a value of about  $1.5-1.6 \times 10^{-9}$  m<sup>2</sup>/s, at 20°C, since the percentage of alcohol is restricted to within a narrow range of 12-12.5 % v/v in this emblematic hydro-alcoholic sparkling beverage. Molecular dynamics simulations were also performed in a wide range of champagne temperature, namely from 4 °C to 20 °C. As expected, the diffusion coefficient of CO<sub>2</sub> was found to increase with increasing temperature.

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# ATTITUDES TOWARD WINE CLOSURE: A COMPARISON BETWEEN SCREW CAPS AND SYNTHETIC CORKS

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**Key Words:** Wine closure, screw caps, synthetic corks

This research is focused on the comparison between two types of wine closure: screw caps and synthetic corks. As these two types of wine closure came as an answer to the problem of the trichloroanisole, a compound responsible of a degraded wine flavour that is partly attributed to natural corks [1], it seems that they're not equivalent in terms of attitudes and acceptability [2]. Effectively, several studies reveal that, on one hand, people tend to perceive screw cap closure as being low quality [3] while on the other hand, synthetic corks seem to have a substantial negative influence on choice [4]. However, most of these comparisons were realised in various countries such as the United States of America or Australia and this question seems, to our knowledge, to have raised little interest in the literature regarding the French context. Consequently, on the basis of a sample of 100 French participants, we first realised independent measures of knowledge, attitudes and acceptability toward screw caps and synthetic corks and then, direct comparison between these two types of wine closure. The results will be discussed from the perspective of the recommendations that can be delivered to wine producers.

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Le Big Data dans sa dimension informatique peut être la source d'une modification des relations entre vendeurs et acheteurs grâce à l'interprétation qu'il permet des connaissances et des émotions de l'individu, de création de valeur dans le E-commerce (avec en particulier une modification potentielle des conditions de formation des prix). De plus, le contrôle et l'extraction des informations par les vendeurs modifient le degré de concurrence entre ceux-ci car si rapidement Internet a révolutionné le marketing en le dotant de nouveaux outils et en enrichissant la connaissance client, jamais les entreprises n'ont disposé d'autant de données sur leur environnement. Elles sont aujourd'hui face à un énorme défi pour les exploiter.

Le rapide développement du Big Data devient une problématique centrale au sein des organisations, et en particulier au sein des directions marketing, des services "commercial" et "client". En effet, le Big Data suit à la trace le consommateur dans sa vie quotidienne notamment au travers de son surf sur Internet. Les données traitées automatiquement accouchent d'informations inédites, issues du croisement de données hétérogènes (géolocalisation, abonnement, niveau de revenu, etc.).

La question de la transparence de l'information est aussi importante : si le E-commerce voit les coûts de recherche d'information diminués (particulièrement avec les sites comparateurs ce qui incite à la diminution des prix), en revanche des effets pervers de la transparence apparaissent en raison de la forte hétérogénéité des prix. Ainsi les commerçants obtenant par le web des informations détaillées sur le consommateur, ceux-ci peuvent plus facilement personnaliser l'offre et donc ensuite accroître la dispersion des offres et des prix. D'où : l'accentuation des phénomènes de discrimination des prix (avec auto-sélection<sup>2</sup>) avec élaboration d'une tarification hétérogène.

**Le monde du vin n'est donc pas à l'écart de ce bouleversement en cours des relations marchandes et sociétales. Notre article visera à repérer dans la pratique, comment s'illustre ou va s'illustrer ce Big Data dans le monde du vin ?**

- Certes la révolution en cours de la relation client est le plus souvent évoquée : par exemple, la prise en compte directe des préférences du consommateur en matière de vins et son impact sur ses achats de vin (d'où déjà : la multiplication des sites sensés faciliter le choix d'un vin et/ou l'alliance vins/nourriture)
- Mais il ne faut pas non plus ignorer l'impact sur la concurrence entre les négociants et leur stratégie des prix grâce à la mise en place de logiciels de prévisions de récolte.
- Les effets dévastateurs sur la réputation d'un vin de certaines informations déposées sur les réseaux sociaux comme la rapide modification des conditions de cotation des vins les plus prestigieux, voire le Big Data au service de l'élasticité des prix sont sans doute encore sous-estimés.
- La potentialité accrue de suivis des conditions climatiques dans les vignes ne doit pas être réservé à quelques producteurs, etc.

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<sup>2</sup> Discrimination par auto-sélection = basée sur les informations obtenues sur les préférences des acheteurs => l'entreprise propose des prix différents associés à des conditions différentes ce qui conduit à s'auto-sélectionner.

## OENOLOGICAL ASPECTS OF SANGIOVESE GRAPES VINIFIED WITH OTHER NATIVE VARIETIES

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**Key words:** : Cofermentation, wine colour, native vines.

It was a Tuscan winemaking tradition to mix Sangiovese with other varieties. It's still the opinion of some producers that this practice allows to soften the harshness of Sangiovese and it makes the wine more harmonious.

The white grape musts have a low polyphenol content, mainly represented by hydroxycinnamiltartaric acids. With the maceration, however, it's possible to obtain musts/wines rich in procyanidins and flavans. These molecules have the ability to react with the anthocyanins, forming coloured polymers more stable and less susceptible to oxidation and to discolouration [1-2].

In this study, two wines (vintage 2012), obtained from a Sangiovese blend (85% w/w), respectively with Colorino and Malvasia, were compared with a pure Sangiovese wine. The analysis, in addition to general parameters, have focused on the evaluation of the phenolic profiles and the colour compositions [3,4].

The results obtained in this study indicate that the addition of Malvasia bianca lunga to Sangiovese in vinification resulted in a decrease of the concentration of anthocyanins. The colour intensity, however, is not affected significantly, in this case, by the addition of white grapes in the winemaking process.

The use of Colorino, instead, integrated the anthocyanic concentration, with consequent improvement in the chromatic parameters.

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## PREDICTION OF THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE SYNTHESIS OF FERMENTATIVE AROMAS

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**Keywords:** *Saccharomyces cerevisiae*; fermentative aromas; Box-Behnken design; environmental factors

Most volatile compounds of organoleptic interest for young and white wines are synthesized by yeast metabolism. Their production depends both on yeast strain and on fermentation conditions. The objective of this work was to study the combined effects of temperature, initial assimilable nitrogen and lipid contents on the production of fermentative aromas. Fermentations were performed using Lalvin EC1118<sup>®</sup> strain and thirty compounds (higher alcohols, acids, ethyl and acetate esters) were measured by GC-MS.

To identify the most discriminating factors in the production of each flavor compound and possible interactions between those factors, experiments were carried out according to a Box-Behnken matrix. This experimental design limits the number of experiments while allowing the calculation of the response surface for each studied parameter and therefore estimating the conditions leading to a maximum (or minimum) production of volatile compounds.

In addition, the “weight” of each environmental factor was calculated. We found that nitrogen had the greatest impact on a large number of flavor compounds, but the synthesis of several compounds (isobutanol, ethyl esters) was also highly influenced by the lipid content and temperature. To better assess the impact of strain genetic background and to evaluate the genericity of the results, we compared the aroma production of several other yeast strains under the two extreme conditions predicted by the model (330 mg/L of assimilable nitrogen, 2 mg/L of phytosterols at 20°C and 70 mg/L of assimilable nitrogen, 8 mg/L of phytosterols to 28°C) and leading to the highest and lowest production of esters, respectively.



## **TYROSOL, HYDROXYTYROSOL AND TRYPTOPHOL CONTENTS IN WINE AS AFFECTED BY FERMENTING YEAST SPECIES AND MUST AERATION**

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**Keywords:** *Saccharomyces cerevisiae*; *Candida zemplinina*; bioactive compounds; higher alcohols.

Tyrosol, hydroxytyrosol and tryptophol, three higher alcohols produced by yeast during alcoholic fermentation, are reported to possess several health-enhancing activities, deriving from their free radical scavenging, anticarcinogenic, cardioprotective and antimicrobial properties [1; 2]. Some Authors reported that the final contents of tyrosol, tryptophol and hydroxytyrosol in wine could be influenced by the microbial ecology of the alcoholic fermentation [3]. However, also aeration of musts during alcoholic fermentation could influence the production of higher alcohols in wines [4]. In the present work, the concentration of the three bioactive molecules were determined in wines obtained under laboratory conditions by varying both yeast species (*Saccharomyces cerevisiae* alone or sequential inoculum of *Candida zemplinina* and *S. cerevisiae*) and aeration conditions of grape must during fermentation. Since several Authors, in literature, discussed about fermentative activity and production of higher alcohols by wine yeasts [5], the first part of the study was aimed to describe the kinetics of the experimental fermentations. The values of two kinetic parameters, fermentative vigor (FV48h) and maximum fermentation rate (Vmax), demonstrate that the presence of *C. zemplinina* had a negative effect on fermentation kinetics in both aerated and non-aerated conditions. Moreover, aeration improves significantly the values of FV48h and Vmax.

In the experimental wines, the highest concentrations of tyrosol, hydroxytyrosol and tryptophol were found when grape musts were fermented by *S. cerevisiae* alone, under non-aerated conditions. Two-way ANOVA demonstrated that the type of inoculum and aeration affected significantly tyrosol and hydroxytyrosol concentrations in wine, whereas the type of inoculum resulted the sole variable affecting tryptophol concentration

In conclusion, the concentrations of tyrosol, hydroxytyrosol and tryptophol in wine depend markedly on yeast species and to a lower extent on aeration conditions of must during alcoholic fermentation. Indeed, the dominance of *C. zemplinina* in the first stage of alcoholic fermentation strongly affects the contents of all the bioactive molecules here taken into consideration.

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## **Metabolomics characterization of the chemical evolution of bottled wines**

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Keywords: Wine, aging, metabolomics

In the present study, combined non-targeted metabolomics approaches were used for the analysis of a vertical series of Burgundy wines, representing natural bottle evolution in cellars. The purpose of this study was to characterize in a non-targeted way the evolution of the chemical diversity of white wines after bottling.

FTICR-MS analyses led to a matrix composed of more than 10 000 distinct masses for the different wines from the vertical series. Each of these wines exhibited numerous similarities with all other wines in the vertical, but also specific masses to each vintage. Differences between wines increased during the bottling period, or aging. Specific masses that influenced most this differentiation were extracted, identified and structurally characterized by UPLC-MS and UPLC-MS<sup>2</sup>.

# How subtle can be the "terroir"? Chemistry-related signatures of two "Climats de Bourgogne"

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Keywords: pinot noir grapes, wine, terroir, vintage, FTICR-MS

The chemical composition of grape berries is influenced by various environmental conditions often considered together as representative of a "terroir". If grapes from a given terroir are assumed to reflect this terroir in their chemical compositions, the corresponding wine should also reflect it.

The aim of this work was therefore to reveal the "terroir" expression within the chemodiversity of grapes and related wines, using ultrahigh-resolution mass spectrometry.

Grapes, and corresponding wines from two distinct– though very proximate – terroirs of Burgundy were analyzed over three vintages. Ultra high-resolution mass spectrometry and ultra-high performance liquid chromatography were used as untargeted and targeted approaches to discriminate complex chemical fingerprints for vintages, classes (wines, skins or musts), and terroirs. Statistical analyses revealed that even if vintages have the most significant impact on fingerprints, terroir discriminations are particularly significant in grapes compositions for a given vintage.

Topic of the conference: active compounds and enological processes  
Preference: oral presentation

## **FROM GRAPE TO SPARKLING WINE: ANALYSIS OF GLYCOSYLATED AROMA COMPOUNDS DURING GRAPE PROCESSING & FERMENTATION**

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Key words: Aroma, precursors, yeast, sparkling wine

The flavour of wine and sparkling wine is significantly influenced by glycosidically bound aromaprecursors in grapes, must and base wines [1]. These odorless precursors are liberated by enzymatic activities of yeast during alcoholic fermentation [2] which triggers the formation of the varietal bouquet during the first or second fermentation [3]. Quantitative analysis of glycosylated aromaprecursors allows an evaluation of the aroma potential of a given must or base wine. Furthermore, yeast strains can be assessed regarding their liberation of aromaprecursors.

Glycosylated precursors from juice and wine samples are obtained and concentrated by an automated solid phase extraction (SPE) system. An aliquot was analysed by FT-MIR, while the other underwent enzymatic cleavage. The extracted free aroma compounds were analysed by gaschromatography/mass spectrometry (GC/MS). Application of a large-volume injection allowed the detection of trace amounts without any further concentration step. Overall 40 substances were quantified using stable isotope dilution assay (SIDA) and combined in the following categories: monoterpenes, C<sub>6</sub>-alcohols, benzene derivatives, C<sub>13</sub>-norisoprenoids and volatile phenols.

Extraction and liberation of aroma compounds from glycosylated precursors were monitored during skin maceration of Riesling and Muscat grapes and varying press regimes. Chemical and sensory analysis of Riesling, Muscat and Chardonnay wines fermented by different yeast strains revealed a significant impact on their composition. Even during secondary fermentation, significant changes were observed in sparkling wines due to the application of various yeast strains.

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## DIFFUSION OF OXYGEN IN CORK: KNUDSEN OR FICK REGIME?

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Key words: diffusion, permeation, cork stopper, oxygen transport

During post bottling aging, oxygen transfer occurs through the stopper and can lead to oxidation reactions. Studying diffusion of oxygen through the cork is of a relevant interest. At present, there is still a misunderstanding in the transport mechanism. Some authors claim that oxygen transport follows Knudsen law [1], while others say that diffusion is governed by Fick law [2]. Moreover the limiting step of oxygen transfer is not clearly identified: is it the diffusion through the cells, the cell walls, the lenticels, the plasmodesms...? The aim of this work is to clearly identify which law governs the oxygen transfer and what is the limiting step.

Oxygen transfer through cork wafers was measured using a manometric permeation technique. The experimental device and the procedure used have been described in detail [2]. In the present work, permeation of oxygen was studied at 298 K on 3 mm cork wafers and for various initial pressures of oxygen ( $50 < p < 800$  hPa).

Results show that the diffusion coefficient of oxygen decreases as the initial pressure increases. This means that oxygen transport through cork does not follow Knudsen regime because the Knudsen diffusion coefficient is independent on the pressure. On the contrary, the dependence of the pressure on the diffusion coefficient is well taken into account by the Fick law considering mass transfer as an activated process. Analysis of data shows that the limiting step in the transfer of oxygen is the Fick diffusion in the cell walls and that the oxygen molecules go through a gas phase in large spaces which could be lenticels or more probably the inside of empty cells when moving from one site to another.

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## MECHANICAL PROPERTIES OF CORK: EFFECT OF HYDRATION

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Key words: cork stopper, compression, water, sorption, visco-elasticity

The use of cork as sealing for wine bottles is due to its low permeability to liquids and gases, imputrescibility and good mechanical properties, in particular its remarkable elasticity [1]. We focused in this study on the compression properties of cork along the radial or the tangential direction at 298 K when cork is stored under various relative humidity environments, from 0 to 100 %.

All stress-strain curves were characterized by an elastic region up to approximately 5 % strain, followed by a large plateau up to 60 % strain caused by the progressive buckling of cell walls.

Firstly, it is worthy to note that the direction of compression significantly affects the Young modulus, with a higher value in radial compression, as already reported by Anjos and Pereira [2,3]. This corresponds to the orientation of the lenticels, which reinforces the rigidity of the material when the strain is applied along their growth direction.

More surprising is the effect of water sorption in cork on its mechanical properties. Both radial and tangential direction exhibit the same behavior, characterized by an increase in the Young modulus up to around 50 % relative humidity and followed by a decrease in rigidity. Maximum observed values are  $46.9 \pm 8.1$  MPa and  $22.1 \pm 2.0$  MPa for radial and tangential directions, respectively. Below that point, the increase in water content leads an antiplasticizing effect while above it gives a more classical plasticizing effect. This is in favor of the existence of an amorphous fraction in cork which goes across the glass transition in this relative humidity range.

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**HOW METABOLITE PRODUCTION CAN BE MODULATED IN WINE BY AN  
INTERACTION BETWEEN TWO YEASTS?  
EXAMPLE OF ACETATE PRODUCTION BY *SACCHAROMYCES CEREVISIAE*  
CO-CULTURED WITH *METSCHNIKOWIA PULCHERRIMA***

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Key words: yeasts interaction, non-*Saccharomyces* yeast, acetate production

Previous studies reported that the use of non-*Saccharomyces* yeasts in mixed culture with *S. cerevisiae* in wine fermentation leads to reduce volatile acidity contents, however, their impact on the metabolic pathway involved in acetic acid formation in *S. cerevisiae* have never been investigated. In this context, we compared the acetic acid production kinetics of *S. cerevisiae* pure culture and *S. cerevisiae/Metschnikowia pulcherrima* mixed culture during alcoholic fermentation, in parallel with the study of the expression of six genes involved in acetic acid metabolic pathway using a reverse transcription-quantitative PCR (RT-qPCR) method with relative quantification. The results showed that interaction occurred between the two yeasts reducing acetic acid amount. The mixed *S. cerevisiae/M. pulcherrima* culture at 1:10 ratio produced 37% less acetic acid than *S. cerevisiae* pure culture. At transcriptomic level, we observed, despite the complexity to correlate gene expression levels to the acetic acid kinetic formation, that the expression levels of these genes were significantly affected. We show for the first time that the entire acetic acid metabolic pathway in *S. cerevisiae* can be altered by the presence of a non-*Saccharomyces* yeast.

# **THE INFLUENCE OF WILD YEASTS BIODIVERSITY AND GRAPE PROCESSING ON AROMA COMPOUNDS IN WINES OF THE GRAPE VARIETIES “RIESLING” AND “SPÄTBURGUNDER”**

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Keywords: biodiversity of yeasts, yeast identification by FTIR spectroscopy, grape processing, aroma compounds

About the influence of different enological grape processing methods on the biodiversity of yeasts in spontaneous fermentations and later on in the resulting wines only less data are available. The aim of this project sponsored by the AiF/FEI was to investigate the influence of several grape processing methods like different maceration times, mash heating, mash fermentation on yeast populations and later on the spontaneous fermentations. How are the aroma compounds and the taste of resulting “Riesling” and “Spätburgunder” wines influenced?

To check the influence on yeast populations and resulting aroma compounds by the processing of white grapes, grounded “Riesling” grapes would be compared to approaches with different maceration times of mash of the same variety.

Effects on yeast populations and aromas would be analyzed using the processing methods of mash heating, mash fermentation and the combination of cold maceration followed by mash fermentation for “Spätburgunder” as a red grape variety.

Sample material was taken at different time points of the processing, the fermentation and the resulting wines to analyze yeast populations and typical compounds. For each sampling point 100 randomly isolated yeasts were identified by FTIR spectroscopy. Chemical analyzes of the different aroma compounds were done by FTIR, HPLC, enzymatic measurements, and GCMS. All produced wines were tasted and sensorial described by a tasting panel.

All collected data are the basis to correlate the different grape processings to the occurring yeast populations and the aromas of the spontaneous fermented wines.

With the knowledge of the processes of the several steps of grape, mash and must processing, also the spontaneous fermentations and the possibility to control these steps, it would be an important precondition for the wine industry to make the risks of spontaneous fermentations more calculable and controllable.