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To cite this version:
Guillaume Antalick, Marie-Claire Perello, Gilles de Revel. Esters in wines: new insight through a survey of french wines. 3. International conference Wine Active Compounds 2014, Mar 2014, Beaune, France. 1 p. hal-02795325

HAL Id: hal-02795325
https://hal.inrae.fr/hal-02795325
Submitted on 5 Jun 2020

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ESTERS IN WINES: NEW INSIGHT THROUGH A SURVEY OF FRENCH WINES

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Key words: esters, wine aroma, wine ageing, yeast metabolism

The concentration of esters, their association and interactions with the sensory perception of wine has recently garnered the attention of wine scientists investigating [1-4]. Esters are no longer considered to impact the aroma of young but are important markers of fruity notes of wines of varying age. Considerable knowledge regarding the role of the concentration of esters in wines from a quantitative point of view was mostly been generated in the 1980s but was limited to few compounds, mainly on ethyl esters of fatty acids (EEFAs) and higher alcohol acetates (HAAs) [5-7].

In this context, there was considerable interest to generate a database on the concentration of a variety of esters to improve the knowledge of wine composition. This work aimed to establish a database specific to esters in wines by quantification using HS/SPME-GC/MS [8] of 30 esters in 183 commercial French wines. Wines from 1 to 29 years old, from different types and styles (normal red, red from carbonic maceration, dry white, sweet white, rosé) and origins were analysed.

Higher levels of EEFAs were found in wines made at low temperature (white and rosé) whereas HAAs synthesis was rather favoured in strict anaerobiosis (red by carbonic maceration (RCM), white). On the contrary, ethyl esters of branched acids (EEBAs) and cinnamates displayed higher concentration in wines made with grape skin contact (RCM, red). These results suggest that lipid and amino acids composition of the must as well as the redox balance of yeast metabolism may play a major role in the formation of esters in wines. Moreover, while EEBAs levels increased during ageing at similar rates in white and red wines, hydrolysis reactions were observed only for HAAs in white wines and for ethyl decanoate and dodecanoate in red and white wines. Furthermore, few trends were highlighted in the ester composition of wines originating from different area.

Beyond the fundamental interest in oenology, this database offers potential new research paradigms in grape and wine biotechnology which could lead to relevant applications for the wine industry in the future.

One of the limiting properties of the polymeric material is the permeability of molecules with low molecular weight as O₂ and remains its major brake to general use by the private sector. So, it is generally accepted that accelerated oxidation is unfavourable for the wine evolution. After dissolution into wine, oxygen triggers series of reactions that are responsible of aroma modification, colour and mouth-feel properties. For wine, determining the properties of oxygen transfer through the films used in packaging remains a challenge [1]. Currently, there are no instruments or standard methodologies allowing industries to determine easily the evolution of wine in Bag-in-Box® (BIB). The reference method used at the moment to determine the permeability of a part of the BIB (bag or tap) is the Ox-Tran® from Mocon®.

The aim of this work was to develop an alternative method which can determine the entire permeation of the BIB. Studies were done by using several analytical methods such as wine model solution with acid ascorbic as oxidation index, measurement based on the reduced power (Chapon method) with a tannometer [2] and luminescence-based technique [3]. Therefore, tests were done by using gas/gas and gas/liquid exchanges. These methods allowed us to test different plastics bags and taps. All developed methods are simple, easy to use and allow BIB testing as a whole.


CARACTERISTIQUES DES EXO-POLYMERES PRODUITS PAR **OENOCOCCUS OENI** ADHEREE SUR LE BOIS DE CHENE

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**Keywords:** *Oenococcus oeni*, Exo-polymères, biofilm, chêne

La fermentation malolactique a lieu dans une cuve en acier ou directement dans le fût de chêne, où le vin est élevé. Elle est réalisée dans la plupart des cas par la bactérie lactique *Oenococcus oeni* qui permet une désacidification, une stabilisation microbiologique et le développement des qualités organoleptiques du vin. Puisqu'elle est présente dans l'environnement viti-vinicole, cette bactérie indigène peut déclencher une fermentation spontanée : ses capacités d'adhésion ont donc un impact sur la qualité du vin. Par ailleurs, l'interface entre le bois et le vin est le siège de transferts de composés du bois vers le vin et inversement de sorption de composés du vin par le bois [1,2]. Or, le développement d'un biofilm à l’interface entre le bois et le vin peut éventuellement favoriser ou interférer les transferts de molécules aromatiques. La matrice du biofilm est constituée d’exo-polymères saccharidiques, nucléiques et peptidiques, ainsi que des enzymes. Ces enzymes catalysent la réaction de transformation de molécules du bois en molécules d’intérêt organoleptique (comme la vanilline) [3]. Ainsi, une meilleure connaissance du développement et de la composition de ces biofilms est importante afin de mieux maîtriser le processus de fabrication du vin.

Après avoir analysé les propriétés d’adhésion et la cinétique de développement des souches de *O. oeni*, notre étude se focalise sur l’analyse chimique des exopolysaccharides, par spectroscopie infra-rouge pour caractériser la structure chimique à la surface et par chromatographie pour identifier les unités monomériques.

Traditionally SO\textsubscript{2} has been added to wine as an antimicrobial and stabilization agent. Since the 25\textsuperscript{th} of November 2005 European legislation has mandated the systematic declaration of wines containing more than 10mg/l of sulfite. But how is this amount really determined in practice and with what accuracy? The problem is that SO\textsubscript{2} undergoes multiple reactions in wine, combining with a number of other compounds. The concentration of the different co-existing or linked species is particularly dependent on the temperature, acid and alcohol content of the wine. Interaction with the wine sample during the analytical procedure itself could influence the results, for both the direct (chromatography, colorimetry, iodometry) and indirect (steam distillation, titrimetry) methods. None of them provides a universal panacea: In some of them, interferences could bias the results, in others, the experimental conditions are so rigid that it is difficult to control and reproduce them correctly (robustness). Even the official method by Franz-Paul (RCE 2676/90, OIV-MA-AS323-04A) is not always clear or precise enough, and leads to confusion or inconsistency between laboratories, especially for the determination of the free form of SO\textsubscript{2}. Agroscope tested the influence of various analytical parameters on the SO\textsubscript{2} results and presents some recommendations, particularly with regard to the temperature of the samples during analysis.
EFFECTS OF REDOX PERTURBATION ON THE SYNTHESIS OF FERMENTATIVE AROMA DURING ALCOHOLIC FERMENTATION

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Keywords: \textit{Saccharomyces cerevisiae}; redox balance; wine fermentation; aroma compounds

Wine alcoholic fermentation is a complex and dynamic process in which the yeast is subjected to multiple stress conditions. \textit{Saccharomyces cerevisiae} wine yeast strains have been selected from the environment for specific technological traits such as high fermentation rate and formation of metabolites. Redox homeostasis is a fundamental requirement for sustaining metabolism. The objective of this work is to gain a better understanding of the effect of the redox balance on the synthesis and regulation of volatile metabolites that contribute to the sensory profile of wine. The important molecules include esters, higher alcohols, volatile fatty acids and volatile sulphur compounds.

To study how the aroma network responds to redox perturbations, we used yeasts overproducing a native NADH- or engineered NADPH-dependent butanediol dehydrogenase catalyzing the reduction of exogenous acetoin into 2,3-butanediol (Ehsani et al, 2009). We found that an increased NADH or NADPH oxidation markedly changed the production of fermentative aroma. Surprisingly, changes in the NADPH demand triggered a similar but weaker response, suggesting inter-conversions between NADH and NADPH cofactors. The production of volatile compounds from Ehrlich pathway in response to increased NADH showed different profiles depending on the leucine or valine pathway degradation, suggesting different metabolic controls. Moreover, a combined increase in the synthesis of propanol and its derived acid and esters was observed with an increased NADH demand, consistent with a redirection of carbon flux towards threonine formation. Changes in the ethyl esters profile was also observed as a result of modifications of NADH and NADPH oxidation, in line with a decrease in the synthesis of their precursors that involves NADPH-consuming reactions (fatty acids).
"Le vigneron et le processus d'élaboration du vin, une approche ethnologique"

Véronique BOIDRON
Anthropologue

Session 2 oral

Je me propose d’exposer, grâce aux méthodes d’enquêtes ethnologiques, une approche inédite du processus d’élaboration du vin centrée sur le vigneron et son univers de travail.

D’un point de vue diachronique, les principes œnologiques ont peu à peu pénétré cet univers durant la deuxième moitié du XXe siècle, permettant notamment d’améliorer les qualités gustatives du produit et de répondre à des normes sanitaires, légales, commerciales, etc. Cela s’est traduit par le développement d’une maîtrise technique autour du vin, mais également par l’arrivée de l’œnologue qui a peu à peu investi cet univers.

Or aujourd’hui, face aux apports des innovations techniques et des découvertes scientifiques dont l’œnologue est un des vecteurs essentiels, certains vignerons mobilisent des savoirs et des savoir-faire qui empruntent au registre de l’empirisme et de l’expérimentation, mettant ainsi à distance des procédés qu’ils jugent trop technologiques. Se pencher sur les représentations singulières associées à ces pratiques permet de saisir les « liens » qui unissent le vigneron à son produit. Dans cette approche que le vigneron qualifie parfois lui-même d’instinctive, et qui peut d’emblée paraître irrationnelle, se révèle en fait un mode de pensée où le vin est conçu dans un processus réciproque de création, en lien étroit avec le travail à la vigne, les caractéristiques du terroir ou des parcelles, le ressenti, la personnalité et l’histoire du vinificateur : ce dernier crée le vin, mais en retour le vin initie et façonne le vigneron et ses savoirs au fil des millésimes.

Ainsi, en marge de la conformation à des normes sociales et à des pratiques techniques incontournables, ces façons de penser et de faire le vin traduisent, pour ces vignerons, une volonté de réappropriation et de maîtrise tant au niveau des procédés d’élaboration que du goût du vin, mais elles reflètent aussi une façon de concevoir leur métier centrée sur une relation intime et forte à la nature, à la vigne et au vin.

**Mots clés** : vigneron, vin, savoir, représentation
There are increasing evidences [1, 2] indicating that some wine aroma compounds form complexes or adducts with different matrix elements, which can deeply affect to their real distribution to the headspace and hence to the aroma perception. The process can be schematized as:

\[ \text{Aroma gas} \leftrightarrow \text{Aroma liquid} \leftrightarrow \text{Aroma complexed} \]

In wine these effects are particularly important for carbonyls which form adducts with SO\(_2\) [3]. In spite that the existence of bisulfite adducts between SO\(_2\) and carbonyls was documented long time ago, and in spite of the fact that some carbonyls, namely methional, phenylacetaldehyde, diacetyl and β-damascenone are amongst the most important aroma compounds of wine (the two first responsible for wine aroma oxidation [4]), there are not reports or tools regarding their measurement of their free and complexed forms.

Twenty-four wines saturated with air were stored at 25 °C in an incubator. When they consumed this oxygen or a week later they were analyzed and re-saturated. This process was repeated five times. Every day the oxygen consumption was controlled with Nomasese oxygen meter. Analyses carried out were free and bonded carbonyl compounds, free and total SO\(_2\), colour, total polyphenols index, and redox potential. In order to have more information about the formation of carbonyl compounds amino acids and alcohols have been analyzed in the original sample and in the final step.

The simultaneous determination of free and bonded carbonyl compounds is based on a HS-SPME-GC-MS method using standards which provide constant headspace concentrations not dependent on the wine and are used to estimate concentrations of analytes in the headspaces (free fractions), and surrogates that behave similarly to the analytes and their relative signals to those of the standards are used to estimate the proportion in bonded form.

The method provides that sensory changes are due from a combination of their release from their complexes with sulfur dioxide and to the “de novo” formation of carbonyls.

Funded by the Spanish Ministery of Economy and Competitiveness (Project AGL2010 230183).

BIOACTIVE STILBENES AS ALTERNATIVE TO SULPHUR DIOXIDE IN WINEMAKING

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Keywords: stilbenes, antioxidant, sulphur dioxide, quality wine

ABSTRACT

Sulphur dioxide (SO\textsubscript{2}) is one of the most versatile and efficient additives used in winemaking due to its antiseptic and antioxidant properties. However, due to the health-related problems that have been associated with SO\textsubscript{2} use, its uses has progressively reduced in winemaking. In the present work a stilbene extract (STE, provided by ACTIchem, Montauban, France) was used as an alternative to SO\textsubscript{2} in microvinifications. Sauvignon blanc and Syrah varieties were selected for the assay. Phenolic profile, antioxidant activity and olfctometric profile were determined in wines elaborated with both, stilbene extract and SO\textsubscript{2}. The results show no significant differences in the parameters related to wine quality (pH, total acidity, organic acids, potassium, IPT, IFC), neither in color-related parameters (color intensity, hue, CieLab*) and sensory. Moreover, acetaldehyde content was lower in white wines elaborated with STE than that elaborated with SO\textsubscript{2}.

The results suggest that the use of stilbenes for replacing SO\textsubscript{2} in wine is feasible. Additionally, pharmacological properties of stilbenes support the use of these compounds as complementary nutritional molecules, and therefore the increase of stilbene content in wine gives it an added-value.
THE DEFINITION OF WINE QUALITY PROFILES AS START POINT TO OPTIMIZE THE WINEMAKING PROCESS

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Key words: wine quality, typicity, winemaking process

Many authors dealt with the concept of wine quality. Generally, based on prior literature, quality can be defined from four different points of view: as excellence or superiority; as value; as conforming to specifications; and as meeting or exceeding customer expectations [1]. In addition, in the case of a Protected Designation of Origin (PDO) wine, the global quality has to be integrated by one more point related to the typicity defined as a set of properties of belonging and distinction [2]. These properties are expressed by sensory attributes which are related to the specific area, raw material and winemaking process [3]. In this study, the concept of quality is the outcome of the combination of eligible characteristics, which meet the needs of the globalized market and which are common to all wines (such as acidity, astringency, viscosity, etc.) characteristics and peculiarities (or identity), which reflect the territorial implications of the product (eg, floral aromas, fruity, etc.). Given these considerations, this study has introduced a new methodological approach in which, starting from the identification of wine samples representative of a PDO area, a sensory profile has been defined and the related chemical markers identified. This has allowed the definition of eligible and peculiarity profiles. These profiles represent tools for the control and optimization of the winemaking process which, starting from an eligible and a peculiar raw material, allow to obtain a wine with predefined characteristics. The study was carried out during three consecutive harvests (2009, 2010 and 2011).


Evolution of fluorescent compounds during white wine ageing assessed by a chemometric approach

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Keywords: Emission Excitation Matrices, PARAFAC components, white wine, ageing

White wine presents a pool of fluorescent materials including a large class of families (polyphenolics, vitamins and amino acids). They can evolve independently upon ageing or interact each other and with other fluorescent macromolecules such as proteins. Each molecule is described by a set of excitation/emission wavelength. Therefore, excitation emission matrices (EEMs) have been well used in the description of wine compounds [1]. EEMs can be viewed as genuine fingerprints of molecular and macromolecular composition in wines. In order to describe EEMs, a trilinear decomposition model, PARAFAC [2], is applied to resume the matrix by a fixed number of components that can be related to single or several fluorophores. Such chemometrics has been applied to determine differences in origins and grape varieties of red wines [3].

Fluorescence measurements were performed in a set of white wines with different evolution stages. EEMs were obtained and treated with the parallel factor analysis (PARAFAC) to describe the evolution of fluorescent compounds upon ageing. Results indicated that 3 components, C1, C2 and C3 could explain the differences of all of the matrices with a very good core consistency of 81 %.

ANATOMICAL FEATURES OF *BOTRYTIS CINERA* RESISTANT GRAPE BERRIES, ‘FRONTENAC’ AND ‘FRONTENAC GRIS’

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Synthesis of berry anthocyanins takes place in a mature organ with an organized arrangement of specialized tissues.\(^1\) In *teinturier* (dyer) varieties of grapes, it is known that anthocyanin synthesis is activated in cells across much of the berry tissue. The mature berry of ‘Frontenac’ (*V. riparia* x Landot 4511), is entirely red and has thus been classified as a *teinturier* grape. ‘Frontenac Gris’, which arose from a single-bud mutation of ‘Frontenac’, has an apparent colorless pulp. Nonetheless, the juice color following direct-pressing of ‘Frontenac Gris’ is often pink, which can result in a wine with an undesirable dark amber to orange color. We removed a 2-mm wide cross-section from both ‘Frontenac’ and ‘Frontenac Gris’ and examined them with an epifluorescent microscope. We found that while both varieties contain anthocyanin inclusions in the epidermal cells, they do not contain specialized organs for anthocyanin synthesis in the cells located beneath the epidermis. Nonetheless, red pigment appears within the cytoplasm of these cells in both cultivars. We thus propose that a passive transport mechanism for anthocyanin exists in these varieties. In addition, we looked at the thickness of the cell wall, the number of pores, and the cuticle thickness of these cultivars in order to compare these anatomical features to other cultivars with known resistance to *Botrytis cinerea*.\(^2\) Current research on novel stilbenoid compounds in these varieties may elucidate further knowledge into their pathogen resistance.

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L'éthanal, molécule clé de la stabilité des vins

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Mots clés : éthanal, SO2, levure, bactérie lactique

Le Groupe ICV réalise depuis 2010 le dosage de l'éthanal (ou acétaldéhyde) sur les vins, par voie enzymatique. La méthode originale de dosage que nous avons développée et que nous présentons ici s'appuie sur un mode alternatif de préparation des solutions d'étalonnage, mettant en œuvre une poudre, l'acétaldéhyde ammonia trimer plutôt que la forme liquide, très volatile à température de laboratoire. La stabilité de ces étaçons garantit davantage de justesse et diminue en outre les risques d'exposition à l'éthanal liquide pur, difficile à manipuler et toxique.

Nous nous sommes appuyés sur plus de 600 analyses chimiques de vins de tout type à différents stades de leur production pour réaliser une exploitation statistique des liens entre éthanal et SO2, permettant de s'affranchir dans la quasi-totalité des situations du dosage des combinants secondaires du SO2 tels que l’acide pyruvique et l’alpha-kéto glutarate. Un modèle prédictif du niveau de SO2 libre, issu de ces travaux, exploitant ce dosage de l'éthanal a été développé. Il permet au vinificateur de quantifier l'addition à réaliser en fonction de son objectif de SO2 libre.

Au-delà de cet outil de calcul, le dosage de l’éthanal apporte des informations précieuses au cours de toutes les étapes de la vinification : la molécule est en effet largement impliquée dans des voies biochimiques de production / consommation levurienne et bactérienne, et est un indicateur des pratiques de gestion de l'oxygène et du SO2 en cave. Notre étude a permis de confirmer la cinétique de production pendant la fermentation alcoolique ainsi que le rôle des bactéries lactiques dans sa consommation lors de la fermentation malo – lactique.

Nos travaux élargissent cette approche globale sur les liens entre SO2, éthanal, oxygène dissous, polyphénols, levures et bactéries, en intégrant également la recherche de voies de consommation ou de combinaisons possibles et exploitées de l’éthanal avec d’autres composants du vin.
CHARACTERIZATION OF NOVEL KILLER TOXINS SECRETED BY NON-SACCHAROMYCES YEASTS

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Key words: killer toxins, non-Saccharomyces yeast, Brettanomyces bruxellensis, yeast interactions

Brettanomyces bruxellensis is a well-known spoilage yeast in red wine. A number of antimicrobial compounds can be used to prevent or eliminate the undesired growth of B. bruxellensis. These include sulphur dioxide, dimethyl dicarbonate and chitosan. The use of inactivated ozone, pulsed electric fields, UV radiation has also been proposed. Recently, the use of a cocktail of β-glucanases was also proven to result in the inhibition of B. bruxellensis growth. In this context, a few authors have suggested the use of killer toxins secreted by an array of non-Saccharomyces yeasts. The nature of most of these toxins remains unclear today, but they could be cell wall enzymes. However, although the toxins considered in these studies have been shown to display killer activity against B. bruxellensis, their native producers did often not originate from the wine environment.

In the current study, non-Saccharomyces yeasts isolated from grape juice were screened for killer activity against B. bruxellensis. Two isolates exhibited strong activity and their killer toxins were further characterised. Their killer property was confirmed against various yeast species and strains. The toxins were shown to display a stronger activity on grape juice than on YPD medium. This phenomenon was further investigated. Activity and stability over a range of pH and temperature were tested and revealed the compatibility of these toxins with winemaking conditions. The killer activity of these toxins was also assessed in the presence of varying concentrations of glucose and ethanol in order to determine at which stage of the winemaking process these toxins were active.

Overall, our study shows the isolation of two novel killer toxins secreted by wine non-Saccharomyces yeasts with strong potential as biopreservatives against B. bruxellensis. Future work includes the purification of these toxins and their identification at genetic and protein levels.
Protein Yeast Extract:
Alternative to white wine clarification and stabilisation

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\textbf{Keywords}
Allergenic, clarification, enological yeast, fining agents, proteins, stabilisation, wine

\textbf{Abstract}
Fining consists in the addition of certain substances to wine that are capable to promote flocculation and sedimentation of undesired components. This practice is essential for the wine final quality, since it improves limpidity, stabilisation and organoleptic balancing. The fining agents available on the market are mainly composed by animal proteins as casein, caseinates, gelatin or egg albumin. However, due to their potential risk on the consumers’ health, recent European legislations have already been adopted and particularly, the use of milk proteins will soon need to be declared on the wine labelling. Therefore, innovative, biologic and non-allergenic alternatives should be further investigated.

In this project, our objective is to identify and develop alternative fining agents, obtained from a natural source, already present in wine - oenological yeasts. Indeed, we have already identified two specific yeast protein extracts with the capacity to simultaneously improve white wine clarification and stabilisation. These potential fining agents present similar effects when comparing to other commercial products in terms of both final brilliance and limpidity. Additionally, they were able to cause a smoother reduction of specific polyphenolic compounds and, interestingly, to accentuate the wine varietal aroma and organoleptic balance.

Currently, we are further investigating the impact of those fining agents in terms of long-term stabilisation and testing the process \textit{up-scaling}, to effectively evaluate their potential industrial use.

Altogether, in this work we have shed new light on the application of yeast proteins for white wine clarification and stabilisation. Moreover, this work is important to overcome the health concerns, related with the use of allergenic animal proteins.

This work is supported by the BioClarVino project (Ref. FCOMP-01-0202-FEDER-021576) financed with funds from FEDER through the COMPETE and QREN program and a Ph.D Grant (SFRH/BD/87649/2012) from Fundação para a Ciência e Tecnologia (FCT), Portugal.
ALTERNATIVE MANAGEMENT OF LEES IN THE VINIFICATION OF
SANGIOVESE GRAPES

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Key words: lees, polysaccharides, proteins, winemaking

This study arises from the need to recover by-products of winemaking that can still provide wine active compound as tannins, aromas and polysaccharides\textsuperscript{1}. The alternative management of lees proposed in this study also reduces the amount of by-product to be disposed. The tests were carried out in 2012 at a winery in the Chianti area. An extraVelvet 1.0 system was used for the treatment of total lees. The extraVelvet 1.0 is a steel macerator equipped with scrolls and an automatic temperature control system. Wine on lees (10\% v/v) was maintained at 22 °C and resuspended every two days for 30 min. Samples were taken every 15 days until two months of treatment. Colour, total polyphenol, phenolic fractions, copigmentation index do not change significantly during the treatment. After only one month of treatments, a significant increase of total polysaccharide was observed. The Gelatin Index as well as the Astringency Mucin Index (AMI)\textsuperscript{2} decrease indicating that polyphenols are become progressively less reactive towards proteins. Total proteins significantly decrease and the amount of α- amino nitrogen do not change in treated wines. No flavour deviations were detected. The obtained results showed that the proposed treatment of lees can contribute to the rapid extraction of compounds involved in the characteristic of astringency, body and structure of wine as well as in the colour stability.

RELEASE OF COMPLEXED VOLATILE SULFUR COMPOUNDS UNDER REDUCTIVE CONDITIONS

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Volatile Sulfur Compounds (VSCs): MeSH (methanethiol), EtSH (ethanethiol), DMS (dimethylsulfide), DES (diethylsulfide), DMDS (dimethyldisulfide) and DEDS (diethyldisulfide) together with hydrogen sulfide (H\textsubscript{2}S) show similar sensorial characteristics described as rotten eggs and cabbage [1]. These compounds usually appear during bottle aging giving rise to problems commonly defined as “reduction”. The origin of this problem is so far not known. In this work we investigate the existence of complexed non-volatile forms of these compounds which may be ultimately responsible for their release upon the reductive conditions usually found in a wine bottle.

Twenty-four wines have been incubated under a zero oxygen atmosphere, the oxygen level was controlled by an oxygen meter from Nomasense, and all the samples were prepared in a glove box from Jacomex with oxygen levels under 5 ppm. VSCs, colour, total polyphenols index, free and total SO\textsubscript{2}, redox potential, major and trace compounds, aminoacids, and metals have been measured. VSCs were analized by two procedures, one that quantifies the total amount and the other which measures the free VSCs concentration (headspace concentration). Both VSCs methods have provide us information about the release of mercaptans in bottle conditions for a wide range of wines.

All these data have allowed us to understand reductive processes, mainly the release of H\textsubscript{2}S and DMS, in many different wines with several behaviors under reductive conditions, which represents an important knowledge in order to try to avoid the reduction of the wine when is in the bottle.

PRINCIPAUX COMPOSÉS BIOACTIFS DE VINS ROUGES EN RAPPORT AVEC L’ORIGINE GÉOGRAPHIQUE DES RAISINS ET LA VINIFICATION

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Mots-clés: resvéraltrol, quercétine, vins rouges, origine, macération.

La qualité intrinsèque et la typicité d’un vin est en rapport direct avec un très grand nombre de variables, issues des facteurs génétiques liés aux cépages, du milieu naturel (origine géographique des raisins) et des facteurs technologiques allant de la maturation du raisin aux procédés d’élevage, ce qui rend très complexe l’effort de quantifier l’effet de chaque variable. Et ceci d’autant plus que l’effet relatif de chaque facteur varie selon le millésime. Par ailleurs, les variables chimiques et organoéptiques utilisées dans l’analyse des vins sont aussi nombreuses, d’où la pratique de choisir certaines comme ‘marqueurs de la qualité intrinsèque et de la typicité’. Les principaux composés bioactifs sont considérés comme étant d’excellents marqueurs. Cette approche est particulièrement employée dans la caractérisation de vins issus de nouvelles régions viticoles.

Cette communication apporte de résultats d’une recherche ayant pour but de quantifier les deux principaux composés bioactifs (resveratrol et quercétine) de vins rouges issus de différentes régions viticoles, très écartées entre elles en ce qui concerne la distance géographique, l’incidence et le rôle des variables des facteurs naturels. Les teneurs mesurées ont été corréllées avec certaines variables climatiques des régions considérées et, pour un cépage précis, ont été aussi corréllées avec la durée de la phase de macération dans la vinification en rouge [1].

Les raisins de cinq cépages rouges ont été prélevés de parcelles préalablement choisies dans quatre régions viticoles. La méthode de vinification en rouge a été standardisée pour tous les vins et les données climatiques ont été prélevées de stations climatologiques liées à chaque parcelle considérée. L’un des cépages issu de l’une des régions a été utilisé dans un essai de durée de macération, dans l’objectif de vérifier l’effet de ce traitement sur les teneurs des composés bioactifs. Les composés ont été dosés six mois après les vinifications, par HPLC-DAD. La méthode employée a été validée à partir de deux gradients préalablement testés [2,3], avec colonne C8 VertiSep UPS, 4.6 x 150 mm, 5 mm, dans les conditions suivantes : phase mobile A = eau acidifiée avec acide formique 0.1% v/v et phase mobile B = méthanol 90% v/v avec acide formique 0.1% v/v ; gradient binaire linéaire de 30 minutes ; volume d’injection = 20 mL et débit = 1,0 mL/min.

Les résultats montrent une corrélation très significative entre l’origine géographique des raisins et les teneurs des composés bioactifs des vins. De même, les teneurs des composés mesurés ont été fortement influencées par la durée de la macération dans la vinification en rouge.


Wine quality and its stability during secondary shelf-life (after bottle opening) is often central to consumer acceptability. Despite care in preserving wine quality during production and bottling, oxidative changes after opening are difficult to avoid, and can negatively impact on sensory character \cite{1,2}. While literature on wine oxidation exists, few studies have investigated how wines change after opening, especially due to limitations still existing in reliable instruments for accurate and high-throughput measurements. In this study chardonnay wines from different geographical origins were evaluated by non-volatile and volatile profiling directly after opening and during six weeks of storage. Chardonnay wines were obtained from France, Chili, Australia, South Africa, Argentina, Brazil and New Zealand. Phenolic acid and volatile profiles were assessed by high pressure liquid chromatography (HPLC) and rapid proton transfer reaction mass spectrometry (PTR-MS) respectively. Measurements of total acidity, pH and total polyphenol content were also recorded. Chemometric analysis of phenolic acid profiles and volatile finger-prints allowed for promising classifications of chardonnay wines by geographical origin and could be applied to observe chemical changes during six weeks of storage after opening. This objective analytical characterization of wine could help verify the wine origin and substantiate its change in quality after opening.

Key Words: Wine origin, shelf-life, phenolics, volatiles


THE ROLE OF ANTIOXIDANTS IN XANTHYLIUMSALT FORMATION AS AGING INDICATOR IN WINE-LIKE SYSTEMS

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Key words: Ascorbic acid, Sulfur dioxide, Glutathione, Xanthylum pigments, Oxidation

During the whole vinification process wine compounds take part in chemical reactions that affect the sensory properties. The mayor catalyst for these reactions is oxygen, which is especially damaging white wines. So called antioxidants such as ascorbic acid, sulfur dioxide, and glutathione are used to prevent oxidation. The effects of these antioxidants on the oxidative evolution of wine-like systems containing (+)-catechin and metal ions have been compared by monitoring O\textsubscript{2} consumption, color evolution by CIELab, as well as (+)-catechin and glutathione decrease by LC-DAD/FD. The oxidation product formation especially the degradation of tartaric acid and the subsequent formation of yellowish colored Xanthylum compounds \cite{1,2,3} was investigated by LC-ESI-ToFMS. The results show that the Xanthylum cation formation is favored compared to the proanthocyanidin formation. The addition of sulfur dioxide does not prevent the oxidation of tartaric acid to glyoxylic acid but slows down Xanthylum formation. Using a model system free of tartaric acid Xanthylum cation formation could be observed by ascorbic acid addition resulting in a color shift towards yellow – green and the formation of an unknown peak. Glutathione addition could prevent Xanthylum cation formation but not the (+)-catechin decrease probably due to glutathionyl-(+)catechin adduct formation. The same system with tartaric acid showed that both sulfur dioxide and ascorbic acid could reduce oxidative color changes and Xanthylum cation formation with sulfur dioxide providing the best protection. The addition of glutathione leads to significant increase in Xanthylum formation and a green – yellowish color and could not inhibit carboxymethine-bridged (+)-catechin dimer formation. In comparison to the other investigated antioxidants glutathione is not able to impede oxidative color evolution.


Metabolic profiling of grape canes stilbenoids in grape varieties
grown in the Loire valley.

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Keywords: grape canes, resveratrol oligomers, metabolic profiling.

Grape canes a non-recycled waste of wine industry (1-5 tons per hectare per year) may have considerable potential as a source of bioactive compounds, especially resveratrol oligomers and other polyphenols. Resveratrol and wine polyphenols are known to exert health-promoting effects including antioxidant capacity, cardioprotection, anticancer activity, anti-inflammatory effects, and estrogenic/antiestrogenic properties (Guerrero et al. 2009). Additionally, resveratrol is a major phytoalexin produced by plants in response to various stresses and promotes disease resistance (Chang et al, 2011). Resveratrol oligomers were obtained by purification using Centrifugal Partition Chromatography followed by preparative Thin Layer Chromatography and semipreparative High Pressure Liquid Chromatography. Dimers (Ampelopsin A, t-ε-viniferin), trimer (Miyabenol C) tetramers (Hopeaphenol, Isohopeaphenol and Vitisin B) of resveratrol were purified and the structures were confirmed by \(^1\)H- and \(^13\)C-NMR spectroscopy. The metabolic profiles of grape canes from various grape varieties grown in the Loire valley (Côt, Cabernet franc, Sauvignon and Gamay) were analyzed by Ultra High Pressure Liquid Chromatography coupled with Mass Spectrometry and Diode Array Detection. Multivariate statistical analyses highlighted biomarkers of each grape variety.


IMPACT OF PRESS FRACTIONING ON PINOT MEUNIER GRAPE JUICE COMPOSITION

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Keywords
Grape juice composition, Pinot meunier, press fractioning, proteins, electrophoresis.

Contribution
The separation of different grape juice press fractions is an important step in the production of sparkling base wines. A complete press cycle for this style of wine is a series of pressure increases resulting in a considerable variation in juice composition during the press cycle (1, 2, 3). After alcoholic fermentation, wines obtained from Cuvées (C) and Tailles (T) grape juices also exhibit strong differences for numerous characteristics [1]. Nevertheless, the impact of the press cycle on wine proteic fraction, largely implicated in wine foaming properties, has never been studied with healthy and Botrytis contaminated grape berries. The aim of this study, carried out on Pinot meunier, was to gain a greater understanding of 1) must composition changes all along the 3h30 press cycle (current analyses), 2) differences in grape juice composition (especially protein content) according to the presence or the absence of rotten bunches (laboratory press), 3) correlation between two grape juice compositions obtained with a 8.000 kg pneumatic press and a 6 kg traditional press.

Protein content, as investigated by SDS-PAGE and silver nitrate staining, shows significant changes in protein levels as the pressing progressed. An increase of the protein content is noted during the two first cycles, followed by a regular decrease during the four last cycles. After SDS-PAGE, 9 bands were isolated and studied by LC-MS + Mascot as Search Engine. Identified proteins originated from Vitis vinifera with MW ranging from 20 to 64 kDa. The Vitis vinifera proteins are: class IV chitinase, VVTL1, thaumatin-like protein, putative thaumatin-like protein and several putative uncharacterized proteins. For some of these proteins, the concentration was not the same in the press juice and in the return juice where a decrease is observed.

REFERENCES
NOVEL BIOSENSOR METHOD FOR DETERMINATION OF BIOGENIC AMINES IN WINE

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Key words: biogenic amines, amperometric biosensor, diamine oxidase enzyme

Biogenic amines are low-molecular-weight, nitrogen-containing compounds of biological importance in vegetable, microbial and animal cells. Although they are essential to living organisms, consumption of food containing high amounts of them may have toxicological effects. Considerable amounts of biogenic amines may accumulate in fermented foods like wine. However, the concentration of biogenic amines in wines is highly variable. It depends on conditions like hygiene, temperature, pH and strains used during fermentation. For this reason, the measurement of biogenic amines provides vital information concerning food safety. [1]

The aim of our work was to develop enzyme based amperometric biosensors for the determination of biogenic amines in wine samples. Diamine oxidase (DAO, EC 1.4.3.6) from Pisum Sativum reacts with the most important biogenic amines which can be found in wine, so the total biogenic amine content could be determined with the DAO biosensor. The enzyme was immobilized on the surface of a graphite electrode in redox hydrogel with horseradish peroxidase, Os mediator and poly(ethylene glycol) (400) diglycidyl ether (PEGDGE) as crosslinker. This modified working electrode was used in wall-jet type amperometric cell together with the Ag/AgCl (0.1 M KCl) reference electrode and a platinum wire as counter electrode. The sensor worked in flow injection analysis system (FIA) using a potentiostat (QuadStat 164, eDAQ, USA). The linear measuring range was 0.01-0.5 mM in histamine equivalent (phosphate buffer 100 mM, pH 7.0).

The developed biosensor method was investigated for the analysis of different wine samples. The biogenic amine content was also measured by HPLC method for validation [2, 3].

References:
EXPERIMENTS WITH SULFITE OXIDASE ENZYME – TOWARDS A NOVEL BIOANALYTICAL METHOD FOR SULFITE DETECTION IN WINES

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Key words: sulfite oxidase, enzyme isolation, biosensor

Sulfite is a widely used preservative in food industry. It prevents oxidation and bacterial growth [1]. Sulfite content has a considerable effect on wine quality. For this reason its determination is of vital importance [2]. Biosensors offer a simple, sensitive and selective alternative for the classical sulfite determination methods (e.g. gas chromatography, acid-base titration). A widespread approach in this field is the use of sulfite oxidase enzyme based electrochemical sensors. Utilizing this enzyme in bioanalytical tools, sulfite detection is based on measuring the oxidation current of sulfite, or that of hydrogen peroxide, which is formed during the enzymatic reaction [3].

Our aim was to develop a novel sulfite oxidase based amperometric biosensor for the determination of sulfite in wines. As this enzyme is currently not available commercially, the first phase of the work was the isolation and purification of sulfite oxidase from chicken liver. We modified the isolation published by Kipke et al. (1989) [4]. Fresh liver was chopped and homogenized in chilled acetone, and then with acetone and ether. The “filter cake” obtained was dried (“acetone powder”), and extracted with buffer. It was followed by two precipitation steps with ammonium sulfate. The sample was dialyzed against a buffered solution containing sodium molybdate, before the ion-exchange chromatography step (Armen Spot Flash LC, Macro-Prep High Q cartridge). After each phase, samples were taken, and the enzyme activity and protein content were measured photometrically. Using this method, 20 fold purification was achieved.

Also, extensive work was carried out to develop a measuring setup for the selective measurement of sulfite and hydrogen peroxide, utilizing screen printed carbon electrodes.

1. Li, Y., Meiping Zhao, M. 2006. Simple methods for rapid determination of sulfite in food products Food Control 17, 975–980
Grape maceration and skin contact is one of the first steps in winemaking and it can condition wine aroma profile and flavour. Several studies have shown how the oxidative maceration occurring in machine harvested grape [1,2] can increase the level of thiol precursors and relevant free thiols.

This latter even is particularly important due the extremely high impact these molecules on final wine aroma. Believing that machine harvest alone could be the Holy Grail of thiol production could underestimate the complexity of the pathways available and the lack of knowledge about where these molecules are originating from (only few % of the known pathways are explaining the free thiols found in wine).

This communication wanted to explore to a closer detail on a larger scale the effect of maceration on the production of two known precursors (S-3-glutathionyl hexan-1-ol and S-3-cysteinyl hexan-1-ol) [3]. 19 Mueller Thurgau and 32 Sauvignon blanc were considered and results seemed to be a bit more complex than what was envisaged in the literature.

Increases of the two precursors were observed only in some sample (not all of them as other found), highlighting how the simple oxidation mechanism might not explain completely the de novo formation of thiol precursors in all grape/wine samples.

OENOLOGICAL TANNINS AS A SOURCE OF THIOL PRECURSORS

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Keywords: oenological tannis; thiol precursors; LC-MS

Oenological tannins are a widely used adjuvant in winemaking. They can help stabilising colour, stringency and flavour. European regulation currently allows tannins produced form vegetable origins in winemaking.

Previous researches have already highlighted the contribution that tannins can give to flavour \cite{harbertson2012impact} and aroma \cite{parker2007effect} of wine.

Among the aroma classes identified and investigated in wine, varietal thiols (i.e. 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate and 4-mercapto-4-methylpentan-2-one) are particularly important for their extremely low sensory threshold and highly appreciated aroma descriptors \cite{roland2011distribution}.

In this communication we are presenting the first identification of two known thiol precursors (S-3-glutathionylhexan-1-ol and S-3-cysteinylhexan-1-ol) in oenological tannins \cite{larcher2013first} and the impact of controlled addition of this adjuvant on free thiols in the final wines \cite{larcher2013pre}.

This evidence opens several avenues for an additional application of oenological tannins in the wine industry and shed new light on the impact that oenological tannins might have on final aroma profile.

\cite{larcher2013pre} Larcher, R., Tonidandel, L., Román Villegas, T., Nardin, T., Fedrizzi, B., Nicolini, G. 2013. Pre-fermentation addition of grape tannin to increase 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in wine. Submitted
Contaminant selective filtration in wine by purified vegetable fiber

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Mots clés : résidus, adsorbant, filtration

Les teneurs en résidus de produits phytosanitaires dans le vin, même très largement inférieurs aux limites maximales de résidus (LMR) fixées sur raisin, restent une préoccupation de plus en plus croissante des consommateurs. Face à cette demande du marché, un produit ADFIMAX® (www.adfimax.com) a été développé par un consortium¹ de PMEs et centres de recherche au cours d’un programme de recherche européen.

Ce produit adsorbe de façon sélective les toxines et polluants présents en très faibles quantités dans le vin. L’usage prévu du produit se fait lors de la filtration des vins, en filtre presse ou filtre avec alluvionnage à 1 kg/m², ou incorporé dans une plaque de filtration. Cet adsorbant est particulièrement innovant car il est produit à partir de fibres d’origine végétale.

Le produit a été testé à échelles laboratoire et pilote, dans différents modes d’utilisation (plaque ou en adjuvant de filtration). Le produit a aussi été comparé au charbon et à une plaque de filtration spécifique. Les paramètres suivants ont été évalués sur vins : suivi de filtration, analyses physico-chimiques, teneur en pesticides, évaluation sensorielle. La capacité filtrante du produit a été démontrée, de même que l’absence d’impact sur les paramètres œnologiques classiques. Les analyses des teneurs de pesticides du vin filtré montrent une réduction de la teneur du vin final de l’ordre de 90 % pour le dimétomorphe, boscalid, iprodione. Les pesticides ont été détectés à très haute concentration dans le gâteau de filtration, confirmant la capacité du produit à adsorber spécifiquement ces molécules.

Cette innovation a fait l’objet d’une demande d’autorisation d’expérimentation en France et d’une évaluation selon les codes Food and Drug Administration (FDA) en Allemagne. Les démonstrations en grands volumes débuteront en janvier 2014 afin de confirmer ces résultats en conditions réelles.

¹ Consortium établit dans le cadre d’un projet FP7 avec les sociétés Realyme, Bavik, Bodegas Riojanas, PJH, Carbis Filtration, Eaton et les centres de recherche, IFV, IGV et UCL
Metabolic Interactom of Microorganisms in Wine
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Keywords
Yeast, Malolactic bacteria, Interaction, Wine, FTICR-MS, LC-MS, Metabolomics

Contribution
The project aims to study the metabolic interaction between yeasts and malolactic bacteria. In the winemaking practice, phenotypes of commercial S. Cerevisae strains can be categorized according to whether they stimulate or inhibit the malolactic activity of bacteria. The assumption is that yeast strains, giving birth to diverse metabolic footprinting or exo-metabolome [1], could alter the bacterial metabolism in different ways. Therefore a sequential culture strategy was used: cell-removed media fermented by different yeast strains will be used to start malolactic fermentation (MLF). Meanwhile the metabolic profile of stimulatory strains was compared to less stimulatory ones by studying yeast-fermented media with non-targeted technics (ICR/FT-MS and UPLC-MS). The non-targeted approach was chosen for its ability to provide in a single run, representations of the thousands of compounds that could be involved in yeast-bacteria interaction [2]. The results highlighted two different yeast phenotypes, and chemical compounds discriminating the two phenotypes could be identified as potential biomarkers of interaction with powerful statistical tools. Their structure will be validated by UPLC-MS/MS fragmentations. The exact influence of these biomarkers to the bacterial metabolism will be studied by looking at the kinetics of the exo-metabolome of malolactic bacteria during MLF. The concentration evolution of selected compounds will be used to parametrize a metabolic network model [3]. All of these approaches will improve our knowledge on the impacts of yeast on the metabolic pathways of bacteria.
References


YEAST PROTEIN EXTRACTS AS AN ALTERNATIVE TO CLASSICAL FINING AGENTS FOR RED WINES

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According to regulation (EU) No 579/2012 of the European Union the use of traditional fining agents derived from egg or milk in must or wine has to be declared towards the consumer if detectable protein traces remain in the product. This has encouraged the search for alternative protein sources, like plants or yeasts that are not regarded as allergenic by European law (regulation (EU) No 1169/2011).

Earlier studies stated that yeast extracts could have a potential to clarify red wines [1–3] without giving details of the influence of these fining procedures on the sensory profile of the wines.

Yeast strains of the genus Saccharomyces were selected in this study that showed autolysis when exposed to stress conditions. The selected yeast strains enabled the production of protein extracts by autolysis. Proteins of the extracts had defined fractions of molecular mass above 10 kDa detected by SDS-PAGE which was not demonstrated in the studies mentioned above. These extracts with a low degree of hydrolysis were successful in clarification of red wines. Turbidity and colour intensity of the wines were influenced by fining with yeast extracts in a similar way to wine fined with gelatine used as a reference. Sensory analysis by experienced tasters revealed no meaty notes, characteristic of standard yeast extracts [4, 5], in the wines after fining with the yeast products.

Since yeast protein extracts are now permitted in the European Union (regulation (EU) No 144/2013) it offers a new category of fining products to the wine makers.

Key words: yeast protein fining

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