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Martedì 5 maggio, ore 11.00

2. Maturity and viticultural techniques strongly impact rotundone concentration in red wines from *Vitis Vinifera L. cv. Duras*

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Rotundone is a grape derived aroma compound responsible for peppery notes in red wines. It has been identified at concentrations surpassing its perception threshold in several red international cultivars (Shiraz, Malbec, Pinot Noir and Gamay), in grape varieties grown in the North East of Italy (Schioppettino and Vespolina) and in the South West of France (Duras, Fer Servadou, Négrette, Prunelard and Abouriou). In 2011 and 2012, two separate experiments were carried out in order to determine the effect of maturity and viticultural techniques on the rotundone concentration in red wines from Duras. These experiments were undertaken using a randomized complete block design with three replicates per treatment. In the first experiment, wines were made from grape samples picked at five levels of maturity (from mid-veraison + 30 days to mid-veraison + 58 days). In the second one, four viticultural techniques were investigated around veraison and compared with a control treatment: 100% fruit zone leaves removal, 40% grape thinning, jasmonic acid spraying and 4 irrigations of 10 mm of rain. Results show that the dynamic of accumulation of this compound is dependent on the vintage conditions and that higher concentrations in wines were reached from half-veraison +44 days. Exogenous jasmonic acid spraying and grape thinning didn't show any significant impact on the rotundone concentration in experimental wines. Leaf removal strongly reduced the rotundone concentration in wines ($-69.0\% \pm 10.3$ and $-52.4\% \pm 6.6$ in 2011 and 2012, respectively) which lead to think that its synthesis is impacted by the level of lighting and/or more likely by the surface temperature of the grapes. On the other side, wines from the irrigated treatment showed higher levels of rotundone ($+29.1\% \pm 21.9$ in 2011 and $+38.3\% \pm 23.8$ in 2012). More generally, vine water status over the veraison–harvest period was identified as a key variable that was well correlated with observed intervintage and intraplot variabilities. These results provide key and practical elements to manipulate peppery aromas in Duras wines. It might also assists growers producing Shiraz, Pinot Noir, Gamay and other varieties where rotundone makes a significant contribution to wine aroma.

Giovedì 7 maggio, 13.20

10. Protease mixture as a viable haze preventing strategy in white wines

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Protein haze is one of the key instabilities in white wine production and is the result of the slow protein denaturation followed by their aggregation into insoluble particles that make the wine appear hazy. Bentonite is commonly used to remove the grape proteins responsible for haze formation, but is associated with significant processing and environmental costs. Proteases potentially represent an alternative to bentonite, but until now none has shown satisfactory activity under winemaking conditions. Proctase, a mixture of Aspergillopepsins I and II, is proposed as a viable bentonite alternative. It is food grade, well characterised and inexpensive, active at wine pH and at high temperatures (60–80 °C). When added to clarified grape juice and combined with short-term heating (75 °C for 1 min), Proctase has shown excellent results in removing haze-causing proteins (80-90% total protein reduction). Experiments have been conducted at laboratory, pilot and commercial scale across a range of juices. Sensory and chemical characteristics of wines made from Proctase-treated juice have not shown any significant differences when compared with bentonite-treated controls. In addition, the cost of Proctase treatment has been shown to compare favourably with traditional batch bentonite treatments. Proctase is the first practical, economically viable alternative to bentonite and its use in Australia and New Zealand wineries has just been approved.

Mercoledì 6 maggio, 13.15

11. The hazing potential of different *Vitis Vinifera* thaumatin-like proteins

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Grape thaumatin-like proteins (TLPs) play roles in plant-pathogen interactions and can cause protein haze in white wine unless removed prior to bottling. Different isoforms of TLPs have different hazing potential and aggregation behavior. In this work we present the elucidation of the molecular structures of three grape TLPs that display different hazing potential. The three TLPs have very similar structures despite belonging to two different classes (F2/4JRU is a thaumatin-like protein while I/4L5H and H2/4MBT are VVTL1), and having different unfolding temperatures (56 vs. 62°C), with protein F2/4JRU being heat unstable and forming haze, while I/4L5H does not. These differences in properties are attributable to the conformation of a single loop and the amino acid composition of its flanking regions. The availability of structural information on haze forming proteins could result in the identification of viable enzymes for the prevention of wine hazing, resulting in an important change in the winemaking stabilization practices.

Mercoledì 6 maggio, ore 17.40

14. Autolysis induction in *Saccharomyces bayanus* by using high-pressure homogenization

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Yeast derivatives are used in winemaking for several applications, such as the release of polysaccharides and mannoproteins; they are obtained by inducing yeast autolysis by thermal or enzymatic treatments. Despite their ability to improve wine colloidal composition, the main limitation connected to their use during wine ageing is due to the release of off-flavors, i.e. volatile molecules produced as a consequence of proteolytic phenomena and the thermal treatments that occur during their manufacturing (induction of Maillard reaction). High pressure homogenization (HPH) is a non-thermal system, used to promote the disruption of microbial cells and the recovery of intracellular components. In the present study, HPH was used for inducing autolysis in a commercial strain of *S. bayanus*. The release of soluble compounds in model solution (proteins, glucidic colloids, amino acids), the effects on cell viability, and the volatile composition of the autolysates were investigated, in comparison with a common thermolysis protocol. HPH seemed a promising technique for promoting autolysis in wine yeasts. The release of soluble compounds (e.g. glucidic colloids) was increased by HPH and it was comparable to that promoted by thermolysis. Moreover, HPH gave a more interesting volatile composition after processing: higher concentration of ethyl.

Induzione dell'autolisi in *Saccharomyces bayanus* mediante omogeneizzazione ad alta pressione

I derivati di lievito sono utilizzati in enologia per diverse applicazioni, come il rilascio di polisaccaridi e mannoproteine; sono ottenuti inducendo l'autolisi del lievito per via termica o enzimatica. Nonostante la loro capacità di migliorare la componente colloidale del vino, la limitazione principale al loro utilizzo in affinamento è dovuta al rilascio di *off-flavors*, molecole volatili prodotte in seguito a fenomeni proteolitici e ai trattamenti termici condotti durante la produzione (induzione della reazione di Maillard). L'omogeneizzazione ad alta pressione (HPH) è un sistema non-termico, utilizzato per promuovere la distruzione microbica e il recupero di componenti intracellulari. In questo studio, l'HPH è stata utilizzata per indurre l'autolisi in un ceppo commerciale di *S. bayanus*. Il rilascio di composti solubili in soluzione modello (proteine, colloidii glucidici, amminoacidi), gli effetti sulla vitalità cellulare e la composizione in sostanze volatili degli autolisati, sono stati studiati in rapporto ad un comune protocollo di termolisi. L'HPH è apparsa una tecnica promettente per indurre l'autolisi. Essa ha determinato un incremento del rilascio di composti solubili (es. colloidii glucidici), paragonabile a quello dato dalla termolisi. Inoltre, ha portato ad una composizione volatile, più interessante: una maggior concentrazione di esteri etilici (note fruttate) e meno acidi grassi (potenziali *off-flavors*). Dopo opportuna ottimizzazione, questa tecnica potrebbe essere utilizzata per la produzione di derivati di lievito appositamente strutturati per l'affinamento dei vini, ma anche per altre applicazioni enologiche.

Giovedì 7 maggio, ore 13.40

17. Oak Barrel and Oxygen: Comparisons, Facts, and Hypotheses

Nevarés Ignacio, Del Alamo-Sanza María - UVaMOX - Universidad de Valladolid

Oxygen directly or indirectly determines the phenomena experienced by wine during barrel aging. The published works up to now do not establish definitive conclusions about how an oak barrel works. These last years our research group UVaMOX has focused its research efforts on the study of different aspects that determine the entry of oxygen into the wine in barrel. We analyzed the kinetics of oxygen permeability in new oak barrels for 1 year and established a non-steady annual oxygen transmission rate of a barrel. So that after approximately 2 months, the oxygen received by the wine in a barrel represents up to 40% of the total oxygen that will be received in a year and up to half of this oxygen can diffuse throughout the oak wood. We used oxygen-imaging technology to study oxygen dynamics in wood which enabled the visualization of oxygen transport through water saturated humid wood, confirming that French oak wood is permeable to oxygen. Besides, we filmed the formation of the headspace in a barrel from the interior while monitoring what vacuum originated for each kind of oak wood. The results show that the classification of rough stave wood in cooperage has indeed allowed the construction of barrels with high and low oxygen permeability. Moreover, it was confirmed that the toasting and bending processes are important in the oxygen permeability decrease in oak wood used for barrel making.

La Barrica de roble y el oxígeno: Comparaciones, Hechos e Hipótesis

El oxígeno determina el proceso de envejecimiento del vino en la barrica. Los trabajos de otros autores no han permitido establecer conclusiones definitivas que describan el funcionamiento de la barrica. El grupo UVaMOX ha centrado sus esfuerzos de investigación de los últimos años en los aspectos que determinan la entrada de oxígeno en la barrica. Los resultados obtenidos son realmente innovadores y con gran repercusión en el sector, ya que se ha evaluado la tasa de entrada de oxígeno en diferentes barricas, encontrándose que lejos de ser constante la TEO cambia con el tiempo de forma que en los primeros 2 meses la barrica ha dosificado al vino hasta el 40% del oxígeno total y que hasta el 50% puede entrar por la propia madera. El empleo del análisis de imagen ha permitido la visualización del transporte de oxígeno a través de madera húmeda saturada de líquido, lo que confirma que madera de roble francés es permeable al oxígeno en esa situación. Además se ha visualizado la formación del espacio de cabeza y la formación de vacío interior en función del origen de la madera. Con todo lo anterior se han construido barricas de alta y baja permeabilidad al oxígeno en base a la clasificación de las preduelas en tonelería, confirmándose que los procesos de domado y tostado de las duelas reducen la permeabilidad al oxígeno de madera de roble.

Giovedì 7 maggio, ore 14.20

19. Understanding and managing reduction problems

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It is widely thought that compounds responsible for reductive aroma deterioration (mainly SH₂ and methanethiol) are formed along bottle storage from chemical reduction of different precursors (mainly elemental S and disulfides). Moreover, highly reputed technical assessors suggest that a major source of the problem is an inadequate handling of oxygen and copper along winemaking, which would provoke by oxidation the formation of the precursors. Recent data indicate that things can be quite different.

In the case of SH₂, it has been demonstrated that this compound does not precipitate with copper salts, as normally believed, but that it forms a reversible complex with Cu²⁺ that largely remains in the wine [1]. Our data confirm that wines at bottling can in fact contain from 10 to more than 100 ppb of complexed SH₂. In relative terms, up to 99.9% of the total SH₂ content of the wine can be under the form of complexes. Furthermore, data show that during anoxic storage there is a spontaneous release of free SH₂ from those complexes, most likely caused by the chemical reduction of copper. Such release accounts to more than 90% of SH₂ accumulated in red wine storage, and to more than 60% in the case of whites and rosés, in which “de novo” formation of SH₂ is more relevant. The rate at which a wine releases SH₂ is positively related to the tendency of the wine to reach more negative redox potentials and negatively related to its copper content. MeSH is also released from Cu-complexes, but its “de novo” formation accounts to around 50% of the total accumulated during wine storage.

These results demonstrate that copper fining delay but do not solve the problem and should not be recommended for wines intended to spend long periods in the bottle. In contrast, it is demonstrated that micro-oxygenation at low oxygenation rates is efficient at reducing total levels of SH₂ and MeSH, while closures with controlled oxygen permeability are efficient at preventing the accumulation of free levels of SH₂ and MeSH. A new rationale for dealing with reduction problems is proposed.

Comprender y manejar los problemas de reducción

Hoy día se piensa que los compuestos responsables de los problemas de reducción (SH₂ y metanotiol, mayormente) se forman a lo largo del envejecimiento en botella por reducción química de precursores (azufre elemental y disulfuros). Asesores muy reconocidos sugieren que el problema es consecuencia de un manejo inadecuado del oxígeno y del cobre en vinificación, lo que causaría la formación de los precursores por oxidación. Nuestros resultados indican que las cosas son bastante distintas.

En el caso del SH₂, se ha demostrado que este compuesto no precipita con sales de cobre en los niveles encontrados en el vino, sino que forma complejos reversibles con Cu²⁺ que en buena parte permanecen en el vino [1]. Nuestros datos confirman que los vinos en el embotellado tienen desde 10 hasta más de 100 ppb de SH₂ en forma de complejos. Hasta el 99,9% del SH₂ total contenido en el vino puede estar en forma de complejos. No solo eso, durante el almacenamiento del vino en anoxia hay una liberación espontánea de SH₂ libre procedente de la rotura de los complejos por posible reducción del Cobre. Tal liberación es responsable de más del 90% del SH₂ acumulado en el almacenamiento del tinto y de más del 60% en el caso de blancos y rosados, en los que la formación de SH₂ “de novo” es más intensa. La velocidad a la que un vino libera SH₂ está

positivamente relacionada con su tendencia para alcanzar potenciales redox más negativos y negativamente con su contenido en cobre. El metanotiol también se libera de sus complejos con cobre, aunque en este caso la formación de novo supone alrededor del 50% del total acumulado durante el almacenamiento.

Estos resultados demuestran que el tratamiento con cobre retrasa pero no elimina el problema y que no debe practicarse en vinos que deban pasar tiempos largos en botella. Por el contrario, la micro-oxigenación a bajas dosis es eficiente en la reducción de los niveles totales de SH₂ y MeSH, y el uso de tapones con permeabilidades al oxígeno controladas, es eficiente evitando la acumulación de SH₂ y MeSH libres. Se propone por tanto, una nueva sistemática para la gestión de los problemas de reducción.

Martedì 5 maggio, ore 11.20

22. Relationship between hyperspectral indices, agronomic parameters and phenolic composition of *Vitis Vinifera* cv Tempranillo grapes

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Predicted scenarios for global environmental change suggest alterations in the grapevine water status that can affect agronomic parameters and the synthesis of phenolic compounds in grapes, affecting the organoleptic properties of wines.

In this work, 15 locations of a vineyard were selected. Grapes samples were collected at harvest time. The detailed phenolic composition of grapes was determined and correlated to hyperspectral indices, plant parameters and soil water status. Hyperspectral indices were calculated from reflectance spectra of both grapevine and bunches. These indices are related to plant canopy structure and vigor (e.g. NDVI, SAVI), to pigment contents (e.g. CNDI, Greenness index), and to water status. Plant parameters (leaf area index, chlorophyll and plant water content), hyperspectral and soil moisture measurements were taken throughout the vegetative growth.

Results showed significant correlations between grape phenolic composition and some hyperspectral indices. Specifically, indices related to vigor calculated for bunches at veraison time showed good, negative correlation with skin phenolic composition total anthocyanins, total flavanols and total hydroxybenzoic acids. Comparing the indices calculated for grapevine at harvest time, negative correlation with skin flavanol content was found. Among the plant parameters, leaf area measured at harvest time showed good negative correlations with flavanol and hydroxybenzoic acids content of grape skins. The flavanol content of skins also showed a negative correlation with the water status of soil. These results nicely outline the potential of the use of hyperspectral parameters for estimating grape phenolic composition before harvest.

Relación entre índices hiperespectrales, parámetros agronómicos y composición fenólica en uvas *Vitis Vinífera* cv Tempranillo

Como consecuencia del cambio climático se prevé que se produzcan alteraciones en el estado hídrico del viñedo que afecten tanto a parámetros agronómicos como a la síntesis de compuestos fenólicos, lo que conlleva cambios en la propiedades organolépticas de los vinos.

Para este trabajo, se seleccionaron 15 localizaciones de un viñedo, en las que se recogieron muestras de uva en el momento de la cosecha. Se determinó la composición fenólica detallada de las uvas y se correlacionó con índices espectrales, parámetros de planta y el estado hídrico del suelo. Los índices hiperespectrales fueron calculados a partir del espectro de reflectancia de cada vid y de los racimos constituyentes. Los índices utilizados están relacionados con la estructura de la vegetación y el vigor (ej. NDVI, SAVI), con el contenido en pigmentos (ej. CNDI, índice de verdor) y con el estatus hídrico. Los parámetros de planta (índice de área foliar, clorofila y contenido en agua de la planta), las medidas hiperespectrales, y las de humedad del suelo, fueron realizadas a lo largo de todo el periodo de crecimiento vegetativo.

Los resultados mostraron correlaciones significativas entre la composición fenólica de la uva y algunos índices hiperespectrales. Específicamente, los índices relacionados con el vigor, calculados para los racimos en el envero, mostraron una buena correlación negativa con el contenido total de compuestos fenólicos en el hollejo, con el contenido total de antocianos, con el contenido total de flavanoles y con el total de ácidos hidroxibenzoicos.

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Los índices calculados para la vid, en el momento de cosecha, correlacionan negativamente con el contenido en flavanoles de los hollejos. El contenido en flavanoles de los hollejos, también mostró correlación negativa con el estatus hídrico del suelo. Estos resultados ponen de manifiesto el potencial del uso de estas parámetros hiperespectrales para estimar de forma rápida y no destructiva la composición fenólica de la uva.

Mercoledì 6 maggio, ore 17.00

25. Evolutionary strategies to develop yeast strains producing less ethanol in wine

Valentin Tilloy, Sylvie Dequin - INRA, UMR1083 SPO

Microbial strategies to develop wine yeasts strains with reduced ethanol yield have been explored for 20 years. Although genetically engineered yeasts producing less ethanol have been obtained, the use of non-GMO strategies to reduce alcohol levels in wine is one of the main challenges of modern winemaking. We present an innovative strategy based on the combination of adaptive evolution under selective salt stress conditions and breeding that we used to select an evolved wine yeast strain that produce substantially more glycerol and less ethanol than the ancestral strain. The metabolic network of this strain is markedly remodeled. Data obtained in wine fermentations experiments at pilot-scale show that the evolved strain has good fermentation performances and produce wines with up to 1.3% (vol/vol) less ethanol. The wines obtained contain more glycerol and 2,3-butanediol, a polyol having no sensorial impact in wine as well as more succinic acid. By contrast, the level of acetic acid was markedly reduced and the production of other undesirable by-products such as acetaldehyde and acetoin remained very low. The overall characteristics of the wines obtained will be discussed.

Strategies evolutives pour développer des levures produisant moins d'ethanol dans le vin

Différentes stratégies microbiennes ont été explorées pour développer des levures œnologiques ayant un faible rendement en éthanol depuis 20 ans. Bien que des souches génétiquement modifiées produisant moins d'éthanol aient été obtenues, l'utilisation de stratégies non-OGM pour réduire la teneur en alcool des vins représente un des principaux défis actuels. Nous présentons une stratégie innovante basée sur la combinaison d'approches d'évolution adaptative en conditions de stress salin et d'hybridation, qui a permis de sélectionner une souche œnologique évoluée produisant plus de glycérol et moins l'éthanol que la souche ancestrale. Cette souche présente un remodelage important de son métabolisme. Des données de fermentations à l'échelle pilote montrent que la souche évoluée présente de bonnes performances fermentaires et produit des vins ayant jusqu'à 1,3 % (vol/vol) moins d'éthanol. Les vins obtenus contiennent plus de glycérol et de 2,3-butanediol, un polyol sans impact sensoriel, ainsi que davantage d'acide succinique. En revanche, la production d'acide acétique est fortement réduite et celle d'autres sous-produits indésirables tels que l'acétaldéhyde et l'acétoïne reste très faible. Les caractéristiques générales des vins obtenus seront discutées.

Giovedì 7 maggio, ore 14.00

29. New inactivated yeast preparation: an innovative way to protect wines against oxidation during storage

Michel Moutounet , Jean-Michel Salmon - INRA UE 999 Pech-Rouge

White and Rosé wines are known in the whole world for being fruity and aromatic. However, during storage, some wines develop an aromatic defective evolution due to oxidation reactions (1). During oxidative storage or during manipulation of finished wines (2, 3), aldehydes increase significantly mainly due to oxidation of alcohols by a coupled oxidation mechanism involving air oxygen and wine di- and trihydroxyphenols. Another major reaction observed in this type of oxidative wine ageing is acetalization between aldehydes and alcohols or polyols such as glycerol. All these reactions are privileged because of the high concentrations of the reactants in wines. All these oxidised compounds are responsible of the sensory depreciation of the corresponding wines, either by the modification of the aromatic balance, or by the browning of the colour of the final wines (1).

Since now several years, it was demonstrated that during oenological fermentations, superfluous oxygen consumption by viable yeast cells during alcoholic fermentation is observed and related to the operation of non-respiratory oxygen consumption pathways (4), metabolic pathways which appear to be also conserved in yeast lees (5, 6, 7). Wine ageing on lees is a traditional wine-making technique used in many countries. Potential oxygen consumption by lees and more precisely by non-viable yeasts during wine ageing was already shown (6, 7). Such an oxygen consumption by *Saccharomyces cerevisiae* lees was recently proved to be related to a mild oxidation of membrane lipids, and more particularly of yeast sterols by the action of free radicals (8).

Such a potential of oxygen consumption by non-viable yeast cells could be therefore applied for the protection of wines against oxidation during storage and/or ageing (9). We search amongst inactivated yeasts those that maintain an efficient ability to consume oxygen, in order to be able to apply these non-viable yeasts on wines susceptible to oxidation along storage and ageing.

After checking for inactivated yeasts able to efficiently consume oxygen in wines, the best characteristics of this oxygen consumption ability were defined on wines: an instantaneous oxygen consumption rate of about $40 \mu\text{g O}_2 \text{ h}^{-1} \text{ g}^{-1}$, and a maximal capacity of oxygen consumption of about $5 \text{ mg O}_2 \text{ g}^{-1}$. Such inactivated yeasts were therefore applied at oenological doses (about 20 g hL^{-1}) on white wines stored under different conditions (with and without sulphite protection) in different forms of containers for 6 to 12 months. The effect of protection against oxidation was checked by physico-chemical measurements of wine colour and by sensorial analysis of the final wines by an expert panel. All the obtained data suggested that selected inactivated yeasts could represent an innovative solution for the protection of wines against oxidation during storage and ageing, and an excellent tool to decrease sulphite addition in the protection against oxidative evolution of stored wines.

Nouvelle préparation de levures inactivées: une nouvelle alternative pour la protection des vins contre l'oxydation au cours du stockage

La réputation mondiale des vins blancs et rosés repose essentiellement sur leurs qualités aromatiques. Toutefois, au cours de leur stockage certains de ces vins subissent des évolutions aromatiques défavorables dues à des réactions d'oxydation (1). Pendant un stockage en conditions oxydatives, ou pendant la manipulation des vins finis (2, 3),

l'oxydation des alcools (et de l'éthanol) conduit à la formation d'aldéhydes par un mécanisme d'oxydation couplée mettant en jeu l'oxygène de l'air et les di- et trihydroxyphénols contenus dans le vin. Une autre réaction défavorable d'oxydation des vins observée est la réaction d'acétalisation entre les aldéhydes formés et les alcools et polyols du vin comme le glycérol. Toutes ces réactions chimiques sont naturellement privilégiées par les fortes concentrations de ces molécules réactives dans le vin. Tous ces composés oxydés sont responsables de la dépréciation sensorielle et organoleptique de tels vins, soit par modification de leur balance aromatique, soit par l'apparition de brunissement de la couleur des vins correspondant (1).

Depuis maintenant de nombreuses années, il a été démontré que pendant la fermentation alcoolique les levures viables étaient capable d'une consommation importante et superflue d'oxygène, via la présence de voies métaboliques non liées à la respiration (4), voies métaboliques conservées dans les lies de levures mortes (5, 6, 7). L'élevage des vins sur lies est une technique œnologique couramment développée dans de nombreux pays, et durant ces élevages la consommation d'oxygène par ces lies de levures non viables a été également démontrée (6, 7). Une telle consommation d'oxygène par des lies de *Saccharomyces cerevisiae* a été reliée à une oxydation lente et progressive des lipides membranaires et plus particulièrement des stérols par des radicaux libres (8).

Une telle capacité de consommation d'oxygène par des levures non viables pourrait donc être avantageusement utilisée pour la protection des vins contre l'oxydation pendant leur stockage (9). Nous avons donc criblé au sein de préparations de levures inactivées, celles présentant les plus fortes capacités de consommation, de façon à pouvoir appliquer de telles préparations sur des vins sensibles à l'oxydation pendant leur stockage.

Après ce crible, les préparations présentant les meilleures caractéristiques de consommation d'oxygène sur vins ont été caractérisées avec les paramètres suivants : une consommation instantanée d'oxygène d'environ $40 \mu\text{g O}_2 \text{ h}^{-1} \text{ g}^{-1}$, et une capacité maximale de consommation d'oxygène d'environ $5 \text{ mg O}_2 \text{ g}^{-1}$. De telles préparations de levures inactivées ont été ensuite appliquées à des doses œnologiques (environ 20 g hL^{-1}) sur différents vins blancs dans différentes conditions (avec ou sans ajouts de sulfites), mais également dans divers contenants pendant 6 à 12 mois de conservation. L'effet obtenu contre l'oxydation des vins a été mesuré par mesure de l'évolution de la couleur des vins, mais également par analyse sensorielle par un jury de dégustation expert. Tous les résultats obtenus montrent que des levures inactivées sélectionnées sur ce paramètre de consommation d'oxygène représentent une alternative innovante pour la protection des vins contre l'oxydation lors de leur stockage et une excellente aide à la diminution des sulfites pour préserver les vins stockés d'une évolution oxydative.

Mercoledì 6 maggio, ore 13.35

32. The centrifuge decanter: an innovative equipment for continuous must extraction for the elaboration of quality white and rosé wines

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The development of technological equipment for both qualitative and continuous pressing remains a challenge in the enological field. In this sense, the horizontal screw decanter centrifuge, technology firstly experienced for the extraction of thermo-treated musts (1), is now also used to separate the must and marc directly from de-stemmed grapes in white and rosé winemaking (2). This technology is based on the instantaneous separation of the two phases, liquid and solid, by centrifugal force in an extremely short time (few minutes). However, this extraction technology can hardly be conceived in a closed environment (continuous process), and one of the potential limitation raised by potential users remains the risk of high oxidation of the must through the use of the decanter, when applied on some sensitive varieties including white or rosé fresh grapes.

Unlike traditional pneumatic pressing systems, where protection against oxidation of the must during pressing is difficult because asking specific adaptations of the press (total inerting of the cage, or sulfites spraying inside the cage), the major advantage of the decanter centrifuge is the possibility of direct injection of various additives through a metering pump located upstream of the apparatus to control oxidation of the must. In the case of the decanter centrifuge, these antioxidant substances get the opportunity to act just upon the disruption of the grape cell compartmentalization with a very high mixing efficiency. This ability is crucial, because cellular disintegration in the centrifuge decanter puts indeed in contact all the substrates of oxidation: - the native phenolic compounds of the grape, oxygen and the enzymatic polyphenoloxidase activity (PPO) of the grape(3).

This peculiar advantage of the decanter centrifuge technology has been used to test the addition of various antioxidants authorized by the legislation on grape harvest for white and rosé winemaking, by comparison to more conventional pressing technologies (pneumatic press with addition sulfite in the juice collector or injection in the cage).

To determine the degree of oxidation of corresponding musts, a controlled oxidation process was first performed: mean individual grape berries were first pressed in a closed reactor blanketed with nitrogen inert gas in order to obtain a must free of oxidation, and then dosed amounts of oxygen were introduced into this reactor. At each oxygen injection, samples were taken, frozen to prevent further oxidation phenomenon and analyzed by HPLC to assay most of the molecules involved in oxidation reactions, including caftaric acid. Oxygen consumption of a given must can therefore be estimated from the calibration curve of the must obtained in anoxic conditions (4, 5).

The obtained results on several grape harvests evidenced that it is possible to use the decanter centrifuge to obtain very little oxidized musts (at a similar or better level than traditional pneumatic press), thus reaching a quality level for white and rosé winemaking. As a matter of fact, the obtained wines were very appreciated for their color and their organoleptic qualities.

These results open new perspectives for the use of decanter centrifuge extraction for a better control of the intensity and color of rosé wines, but also more generally for a better control (decrease) in amounts of sulfites applied during the phases of prefermentative technological steps.

Le décanteur centrifuge: un équipement innovant pour une extraction continue et qualitative des moûts destinés à l'élaboration de vins blancs et rosés de qualité.

Le développement d'équipements technologiques permettant à la fois un pressurage qualitatif et continu reste toujours un challenge d'actualité. A cet égard, le décanteur centrifuge à vis horizontal, technologie d'abord expérimentée pour l'extraction des moûts de vendange thermo-traitée (1), est désormais utilisé également pour séparer directement moût et marc à partir de vendange égrappée en vinification en blanc et en rosé (2). Sa technologie est basée sur la séparation instantanée des deux phases, liquide et solide, par la force centrifuge en un temps extrêmement court (quelques minutes).

Toutefois, cette technologie d'extraction ne peut que difficilement se concevoir en milieu fermé (process continu), et l'un des freins potentiels soulevé par les utilisateurs potentiels reste le risque de forte oxydation du moût extrait du décanteur, paramètre potentiellement néfaste quand il s'agit de travailler certains cépages sensibles et notamment en vendange fraîche blanc ou rosé.

Contrairement aux pressoirs pneumatiques traditionnels, où la protection contre l'oxydation du moût en cours de pressurage reste difficile car demandant une construction/adaptation spécifique du pressoir (inertage total de la cage, ou pulvérisation de sulfites à l'intérieur du pressoir), l'avantage majeur du décanteur centrifuge est qu'il est possible d'injecter en cours d'extraction, grâce à une pompe doseuse située en amont de l'appareil, divers additifs pour limiter l'oxydation du moût. Ces substances antioxydantes ont, dans le cas du décanteur centrifuge, la possibilité d'agir dès la rupture de la compartimentation cellulaire avec une très haute efficacité de mélange. Cette faculté est primordiale, car c'est lors de cette déstructuration cellulaire que les trois éléments nécessaires à la réaction initiale d'oxydation sont mis en contact : les composés phénoliques originaires du raisin, l'oxygène de l'air et les activités enzymatiques polyphénoloxydase (PPO) du raisin (3).

Cet avantage du décanteur centrifuge a donc été mis à profit pour tester l'ajout de divers agents antioxydants autorisés par la législation sur des vendanges destinées à la vinification en blanc et rosé, par rapport à de technologies de pressurage plus conventionnelles (pressurage pneumatique avec ajout de sulfite à la maie ou en injection dans la cage).

Pour déterminer le degré d'oxydation correspondant des moûts obtenus, une procédure d'oxydation contrôlée a été effectuée en amont : des baies isolées sont pressées dans un réacteur clos et inerté par un gaz neutre tel que l'azote permettant l'obtention d'un moût exempt de toute oxydation, puis des quantités dosées d'oxygène sont introduites dans ce réacteur. Au fur et à mesure de l'injection d'oxygène, des échantillons sont prélevés, bloqués pour empêcher tout phénomène d'oxydation ultérieure et analysés par HPLC, afin de doser les molécules réagissant à l'oxydation, dont l'acide caftarique. La consommation d'oxygène du moût peut donc être estimée d'après la courbe d'étalonnage du cépage réalisée en conditions anoxiques (4, 5).

Les résultats obtenus sur plusieurs vendanges montrent clairement qu'il est ainsi possible d'utiliser le décanteur centrifuge pour obtenir des moûts très peu oxydés (d'un niveau équivalent, voire meilleure qu'avec un pressoir pneumatique traditionnel) donc de très haute qualité pour la vinification en blanc et en rosé, conduisant à des vins appréciés au niveau de leur couleur ou de leurs qualités organoleptiques.

Ces résultats ouvrent de nouvelles perspectives d'utilisation du décanteur centrifuge en extraction pour une meilleure maîtrise de l'intensité et de la coloration des vins rosés, mais également plus généralement pour une meilleure maîtrise (diminution) des quantités de sulfites appliquées pendant les phases de travail préfermentaires.

Giovedì 7 maggio, ore 13.00

39. Application of activated charcoal made from vine shoots (VS) in the wine industry. Relationship between their physicochemical and textural characteristics and the impact on color and aromatic properties of wines

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In the present study, vine shoots (VS) of the cv. Cabernet-Sauvignon were used as the raw material for the preparation of activated carbons. The activated carbons (ACs) were prepared by physical (air, CO₂ or steam) or chemical (H₃PO₄, ZnCl₂ or KOH) activation. The ACs were characterized and valuable information regarding their chemical composition, texture, and surface chemistry was obtained. The ACs exhibits S_{BET} values ranging from 23 up to 435 m²g⁻¹ whereas V_{map} (macropore volume) and V_{mep} (mesopore volume) were from 0.17-0.62 and 0.05-0.24 cm³g⁻¹ respectively. Activated carbons were added to a white varietal wine cv. Pardina in doses of 50 g hL⁻¹ and concentrations of phenolic substances (total polyphenol, flavonoid, hydroxycinnamic acids and brown polymers), chromatic characteristics (IC and TC) and the aromatic composition (alcohols, carbonyl compounds, acetates, esters and acids) were measured before and after the treatment. With the aim to find the most desirable charcoal's characteristics for the in the wine industry, correlations between the parameters analyzed in the charcoals and the impact on chromatic and aromatic properties of wines were analyzed. V_{map} followed by V_{mep} showed the most significantly positively correlation with decrease of brown polymers and IC. On the other hand, V_{mep} was correlated with decrease of alcohols, carbonyl compounds and ethyl esters while VT and V_{map} were correlated with decrease of acetates and volatile acids respectively.

Aplicación de carbones activados preparados procedentes de madera de poda de sarmientos en la industria enológica. Relación entre sus características físico químicas y texturales y el efecto en las propiedades cromáticas y aromáticas de los vinos.

En el presente estudio, se han preparado carbones activados a partir de residuos de madera de poda de sarmientos de cv. Cabernet Sauvignon. Se emplearon las técnicas de activación física (aire, CO₂ y vapor de agua) y química (H₃PO₄, ZnCl₂ y KOH). Los materiales resultantes se caracterizaron físico-química y texturalmente, determinándose la superficie de BET (S_{BET}), el volumen de micro, meso, macro y total de poros (V_{mip}, V_{mep}, V_{map}, VT). S_{BET} de los diferentes carbones estuvo comprendida entre 23 -435 m²/g, y VT entre 0,51-1,1 cm³g⁻¹ respectivamente, en tanto que V_{map} y V_{mep} entre 0.17-0.62 y 0.05-0.24 cm³g⁻¹ respectivamente. Los carbones preparados se adicionaron en dosis de 50 gHL⁻¹ a un vino blanco monovarietal cv. Pardina y se midieron las concentraciones de sustancias fenólicas (polifenoles totales y flavonoideos, ácidos hidroxicinámicos y polímeros marrones), características cromáticas (IC y TC), y la composición aromática (alcoholes, compuestos carbonílicos, acetatos, ésteres y ácidos) antes y después del tratamiento. Para conocer que características de los carbones son las más deseables para el empleo de éstos en la industria enológica se han buscado correlaciones entre los parámetros analizados en los carbones y la incidencia del tratamiento en las propiedades cromáticas y aromáticas de los vinos. V_{map} y en menor medida V_{mep} están significativa y positivamente correlacionados con la disminución de polímeros marrones, y pérdida de intensidad de color, que se pretende obtener mediante el tratamiento con los carbones. En cuanto a la disminución de compuestos aromáticos observada tras el contacto vino-carbón, V_{mep} se correlacionó con el descenso

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de alcoholes, compuestos carbonílicos y ésteres, en tanto que VT y Vmap con el de acetatos y ácidos de los vinos respectivamente.

Mercoledì 6 maggio, ore 17.20

40. Increase of yeast autolysis in sparkling wines through the modulation of *ATG* genes

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In a previous study *Saccharomyces cerevisiae* flocculent strains isolated about 30 years ago from different vitivinicultural areas of Northern Italy were investigated in order to select strains to be used for sparkling wine production. Efficient wine yeast flocculation after primary alcoholic fermentation leads to the formation of compacted sediments, reducing the handling of wines and facilitating wine clarification. However, compacted sediments reduce autolysis process which plays a key role in sparkling wines production. Autolysis in enological conditions is a slow process which corresponds to the dissolution of organelles. Since autophagy is involved in the degradation of damaged organelles it has been recently postulated that it may contribute, at least in part, to the outcome of autolysis.

So, in this study the same *S. cerevisiae* strains were characterized for the autolytic character performing an accelerated autolysis assay in YNB at 30°C for 15 days. The autolysis process was monitored through the determination of free amino acids content and total protein concentration. Moreover the expression of *ATG1*, *ATG17* and *ATG29* genes was evaluated. On the basis of the obtained results 12 strains were selected and the autolysis was monitored in real second fermentation conditions and a relationship between the genetic variation and autolytic phenotype was established. In particular, *ATG29* gene could be proposed as a biomarker for autolysis in flocculent wine strains.

Aumento dell'autolisi in vini spumanti mediante modulazione dei geni ATG

In un precedente studio sono stati analizzati dei ceppi flocculanti di *Saccharomyces cerevisiae* isolati da uve e mosti di diverse aree vitivinicole del Nord Italia al fine di selezionare ceppi da impiegare per migliorare la qualità dei vini spumanti. I lieviti flocculanti hanno la capacità di aggregarsi in ammassi multicellulari sedimentandosi sul fondo della bottiglia favorendo il *remuage* e la sboccatura. Tuttavia i sedimenti riducono l'autolisi. Questo processo influisce su aroma, sapore e sulle proprietà del vino spumante, tanto che vi è una stretta correlazione tra qualità degli spumanti e capacità autolitica del lievito. La capacità autolitica del lievito in condizioni enologiche richiede lunghi periodi. Recentemente, è stato visto che l'autofagia, un processo catabolico comune a molte cellule eucariotiche che coinvolge la degradazione dell'intero citoplasma, può essere messo in relazione, almeno in parte, all'autolisi. L'ipotesi è che lieviti che presentano autofagia deregolata muoiano più facilmente e si abbia un'autolisi accelerata. Pertanto, in questo studio gli stessi ceppi di *S. cerevisiae* sono stati caratterizzati per la loro capacità autolitica in YNB a 30°C per 15 giorni. Il processo autolitico è stato monitorato determinando il contenuto di aminoacidi liberi e proteine totali. Inoltre, è stata determinata l'espressione dei geni *ATG1*, *ATG17* e *ATG29*. Sulla base dei risultati ottenuti sono stati selezionati 12 ceppi in cui l'autolisi è stata valutata mediante microspumantizzazioni. La biodiversità genetica ottenuta è stata correlata al fenotipo autolitico. Inoltre, i risultati ottenuti hanno mostrato che il gene *ATG29* potrebbe essere impiegato come marker dell'autofagia nei ceppi vinari flocculanti.

Martedì 5 maggio, ore 11.40

42. Minimal pruning and severe trimming as two helpful techniques to increase the anthocyanin: sugars ratio in grapes

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A number of cultural techniques have been proposed in order to delay wine grape ripening in the face of global warming. In order to evaluate the effects of minimal pruning (MP) on grapes cultivated in warm areas, a long-term study about minimal pruning on Tempranillo (*Vitis vinifera* L.) was conducted in Badarán (La Rioja, Spain). For every vintage between 1999 and 2013, grapes from minimal-pruned vines and the vines of control treatment were harvested at the same time. On this basis, a further study was initiated in 2014, aimed at comparing berry qualities between the two treatments and at verifying the positive effects of MP on wine industry under global warming. The above mentioned long-term study showed that MP, on average, increased yield (+100%) and reduced the TSS content (-8%) compared with the control treatment. The 2014 study demonstrated that berries from the MP group got a similar level of TSS content as that of the control group 20 days later. At the same level of TSS content, compared with the control group, must from MP group, significantly, had smaller berry size (-35%), smaller cluster size (-60%), larger yield (+77%), smaller leaf-fruit ratio (-32%), more total acids (+37%), higher PH (+6%), more tartaric acid (+13%), less malic acid (-15%). And above all, must of MP group presented higher anthocyanin content (+17%) as well as higher anthocyanin: sugar ratio (+21%). The current results show that MP can effectively delay berry ripening and restore the anthocyanin : sugar ratio, or/and reduce wine alcohol content; The results also support the hypothesis that MP does help to improve berry quality in warm climatic conditions.

Another study was aimed at evaluating the effects of trimming on delaying berry ripening. The intensity of trimming was investigated with 3 treatments carried out: Control (C), Trimming (T) and Double Trimming (DT). The grapes were harvested at the same level of TSS and parameters of must were analyzed. The trimming practice delayed the veraison date significantly. Trimming led to an increase in total acidity and tartaric acid, and a decrease in leaf-fruit ratio, pH and malic acid. The objective of increasing anthocyanin: sugar ratio was not achieved, probably due to the abnormal climate of this vintage.