Codice *Number*	**Titolo** (clicca sul titolo per andare direttamente al riassunto) *Title* *(click on the title to be redirected to the abstract)*
COMPOSIZIONE DEL VINO E QUALITÀ Martedì 7 maggio, Sala Internazionale (A), 17.00 – 19.00	
35	Comparative evolution of dissolved gases and SO_2 during storage of a rosé wine of Cinsault bottled in PET and glass bottles Relatore : Marie Toussaint, INRA UE999 Pech-Rouge, Narbonne, Francia
54	Use of ATR-FTIR spectroscopy to monitor alcoholic fermentations and control the physiological state of yeast Relatore : Silvia de Lamo Castellvi, Departament d'Enginyeria Quimica, Universitat Rovira y Virgili, Tarragona, Spagna
41	Relationship among instrumental texture properties, phenolic composition and postharvest dehydration kinetics of wine grapes Relatore : Luca Rolle, Dip. Scienze agrarie, forestali e alimentari, Università di Torino
39	Assessment of oxidation during winemaking technological processes: a new approach based on the measurement of phenolic compounds in musts Relatore: Jean-Michel Salmon, INRA UE 999 Pech-Rouge, Francia
58	Influence of oxidation mechanisms during must elaboration on the aromatic quality of Melon B. and Sauvignon Blanc wines Relatore : Rèmi Guerin-Schneider, IFVV – UMT Qualinnov, Montpellier, Francia
VITICOLTURA E SOSTENIBILITÀ Mercoledì 8 maggio, Sala Internazionale (A), 17.00 – 18.40	
55	E-CO_2: recovery and re-use of CO_2 from wine fermentations Relatore : Sandra Torriani, Dipartimento di Biotecnologie, Università degli Studi di Verona
50	A cellar of bioclimatic architecture experiments geothermy Relatore : Jaume Gramona Martì, Cavas Gramona e Universitat Rovira I Virgili, Tarragona, Spagna

8	Pinot blanc and Pinot gris arose as independent somatic mutations of Pinot noir Relatore : Silvia Vezzulli, Fondazione Edmund Mach, San Michele a/A
10	Improving Grape Phenolic Content and Wine Chromatic Characteristics through the Use of Elicitors Relatore : Yolanda Ruiz-García, Food Science and Technology Department, University of Murcia, Spain
TECNOLOGIA DI CANTINA Giovedì 9 maggio, Sala Internazionale (A), 12.00 – 13.30	
9	Dismissing enological myths: effect of ethanol and maceration enzymes on grape seed tannin extraction. Enological implications Relatore : Maria Encarnacion Gomez Plaza, Food Science and Technology Department, University of Murcia, Spagna
38	Full automation and control of vinifications by FT-NIR spectroscopy: An innovation presenting ground-breaking opportunities Relatore : Ramón Mira de Orduña, Department of Food Science, Cornell University, NY, USA
40	Electrodialysis, a physical and multipurpose process for an enology of precision: last development, pH adjustment of wines by bipolar membranes Relatore : Magali Bes, INRA UE 999 Pech-Rouge, Narbonne, Francia
31	Recovery of aromatic aglycones from winemaking by-products Relatore : Carolina Muñoz González, Instituto de Investigación en Ciencias de la Alimentación (CIAL) CSIC-UAM, Madrid, Spagna
MICROBIOLOGIA ENOLOGICA Giovedì 9 maggio, Sala Internazionale (A), 13.30 – 15.30	
42	Interspecies gene transfer in wine yeasts: a safe alternative to GMO technology Relatore : Matthias Sipiczki, Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Ungheria
53	Yeast COQ1 gene acts as a geraniol /nerolidol synthase in *de novo* synthesis of terpene compounds in wine production by *Saccharomyces cerevisiae* Relatore : Eduardo Dellacassa, Dep. de Quimica Organica, Seccion Enologia, Facultad de Quimica-UdelaR, Montevideo, Uruguay
26	An effective solution to limit biogenic amines in winemaking: Enzymatic extracts from vineyard fungi able to reduce biogenic amines content in white and red wines Relatore : Carolina Cueva Sánchez, Instituto de Investigación en Ciencias de la Alimentación (CIAL), CSIC-UAM, Madrid, Spagna

43	Study of gene expression: a tool to understand the behaviour of *Oenococcus oeni* in wine Relatore : Cristina Reguant, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, Tarragona, Spagna
6	Identification of phenylacrylic acid decarboxylase (pad) gene of Dekkera bruxellensis involved in formation the vinyl derivatives Relatore : Liliana Godoy Olivares, Departamento en Ciencia y Tecnología de los Alimentos. Universidad de Santiago de Chile
57	New dangers in enology: *Staphylococcus epidermidis* producing histamine in wines Relatore : Yaiza Benavent-Gil, Enolab, Departament de Microbiologia i Ecologia, Universitat de València, Spagna

6 – Identification of phenylacrylic acid decarboxylase (pad) gene of Dekkera bruxellensis involved in formation the vinyl derivatives

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The species of the genus Dekkera have been described as the main spoilage wine yeast. The presence of this yeast in wine is associated with the occurrence of phenolics aromas described as "medical", "horsy", etc. The formation of these aromatic compounds is carried out by the metabolism of hydroxycinnamic acids by two enzymes: coumarate descarboxylase (CD) y vinylphenol reductase (VR). Using bioinformatics analysis, and analysis of the N-terminal sequence and internal peptides of the purified CD protein (Godoy et al. 2008), we have determined sequence of the gene that encodes it. To confirm its functionality we transformed yeast Saccharomyces cerevisiae. Results showed that in the culture medium where was grown the transformed microorganism the production of 4-vinylphenol was higher than control microorganism. Similar results were obtained in the protein extract. This indicated that the cloned fragment correspond the gene that encoded the phenylacrylic acid decarboxylase of D. bruxellensis.
The sequence obtained has a percentage of identity of 43% on average as compared with the nucleotide sequences of ascomycetes reported for this activity (Gibberella zeae, Candida. albicans, Candida guilliermondii and S. cerevisiae) and 34% on average when is compared with bacteria (Bacillus subtilis and Bacillus pumillus). The analysis of in silico translation of the sequence shows that the percentage of identity is on average 8% with PAD protein G. zeae, C. albicans and S. cerevisiae, and 36.7% when compared with the PADCg protein of C. guilliermondii. Thus, the identification of the pad gene, that encoding the first enzyme of training route volatile phenols, will allow the development of technology tools to control the formation of these compounds in wine by D. bruxellensis. Also will be possible to study gene regulation, either at the transcriptional or post-transcriptional level, and if required a minimum concentration of substrate to induce expression.

Resúmen en castillano

Identificación del gen de la ácido fenilacrílico descarboxilasa (pad) de *Dekkera bruxellensis* involucrado en la formación de derivados vinilo
Las especies del género *Dekkera* han sido descritas como la principales levaduras que causan alteraciones organolépticas en vino. La presencia de esta levadura en vino se asocia con la aparición de aromas fenólicos descritos como "medicina", "caballo", etc. La formación de estos compuestos aromáticos es llevada a cabo por la metabolización de los ácidos hidroxicinámicos mediante dos enzimas: una cumarato descarboxilasa (CD) y una vinilfenol reductasa (VR). Usando análisis bioinformáticos, así como análisis de la secuencia N-terminal y de péptidos internos de la proteína CD purificada (Godoy et al. 2008), se ha determinado la secuencia del gen que la codifica. Para confirmar la funcionalidad de este gen se transformó la levadura *Saccharomyces cerevisiae* L1125. Los resultados mostraron que en el medio de cultivo en el que se cultivó el microorganismo transformado, la producción de 4-vinilfenol fue mayor que la de la cepa control. Resultados similares fueron obtenidos al cuantificar la actividad CD en extractos de proteínas, en donde el extracto proteico de la levadura transformada tiene una mayor actividad CD que la levadura control, lo cual se reflejó en la mayor producción de 4-vinilfenol. Estos resultados indican que el fragmento clonado corresponde al gen que codifica para la ácido fenilacrílico decarboxilasa de *D. bruxellensis*.
La secuencia obtenida posee un porcentaje de identidad de 43% en promedio cuando se compara individualmente con las secuencias nucleotídicas de ascomycetes reportadas para esta

actividad (*G. zeae*, *C. albicans, S. cerevisiae* y *C. guilliermondii*) y de un 34% en promedio cuando la comparación se hace con bacterias (*B. pumillus* y *B. subtillis*). El análisis de la traducción in silico de la secuencia muestra que el porcentaje de identidad es en promedio de 8% con la proteína PAD de *G. zeae, C. albicans y S. cerevisiae*, y de un 36,7% cuando se compara con la proteína CgPAD de *C. guilliermondii*.
De esta forma, la identificación del gen que codifica para la primera enzima de ruta de formación de fenoles volátiles, permitirá el desarrollo de herramientas tecnológicas para controlar la formación de estos compuestos en vino por parte de *D. bruxellensis*. Asimismo, permitirá estudiar cómo ocurre la regulación de este gen, ya sea a nivel transcripcional o post-transcripcional, así como también si es necesaria una concentración mínima de sustrato para inducir la expresión.

8 – Pinot blanc and Pinot gris arose as independent somatic mutations of Pinot noir

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In grapevine (Vitis vinifera L.), accumulation and fixing of somatic mutations represent frequent events, allowing growers to select and propagate new cultivars. A great deal of somatic mutations does not affect the entire meristem but only a portion of it (chimeras). Resulting from the layered structure of the meristem, chimeras are composed by two genetically distinct tissue layers placed adjacent to one another. Among spontaneous somatic mutations occurred in grapevine, those affecting the berry colour locus are the most documented. Grape berry colour is due to the presence of a single pigment family, the anthocyanins, which largely vary in concentration and composition depending on the grape cultivar. In many plants anthocyanin biosynthesis is controlled by regulatory genes belonging to the Myb family of transcription factors. Two Myb-related transcription-factor genes, VvMybA1 and VvMybA2, regulate anthocyanin biosynthesis in V. vinifera grapes. Inactivation of these two functional genes, through the insertion of the Gret1 retrotransposon in VvMybA1 promoter and through a non-synonymous SNP present in the VvMybA2 coding region, gives rise to a white berry phenotype. Recently, several genetic and genomic studies revealed that the colour locus is a cluster of four Myb-like genes located on chromosome 2. As one of the founder varieties and cultivated worldwide, Pinot had several chances to undergo somatic mutations. Most of these affected the ancestral black berry colour, and gave rise to cultivars such as in Pinot blanc and Pinot gris. The most established evolutionary model is that Pinot blanc arose from Pinot gris which arose from Pinot noir, even if the relationship between Pinot blanc and Pinot gris has not yet been fully explored. Pinot gris is reported to be a periclinal chimera of Pinot noir, but also in this case the exact nature of the genetic modification remains to be determined.
Our study has questioned this evolutionary model. Taking advantage of a layer-specific structural analysis of the berry colour locus in 4 Pinot noir, 10 Pinot blanc and 13 Pinot gris clones, along with its naturally derived chimeras or sports, we provide an evolutionary explanation of the somatic mutations that have affected the locus of berry colour. Through the study of the structural dynamics along the chromosome 2, a very large deletion (ca. 4,300 Kb) present in a single Pinot gris cell layer was identified and characterized, while a short deletion (ca. 150 Kb) present in both Pinot blanc cell layers was detected. Within the same cultivar, all clones shared the same deletion pattern. As recently observed in other Pinot noir clones, we can suppose that the structural changes that occurred in Pinot gris and in Pinot blanc were stress-mediated, resulting in the activation of a mobile genetic element.
Theoretically, the origin of a colourless berry mutant can be ascribed to two distinct models: i) the sequential model, where the black-skinned berry ancestor gave rise to the grey-skinned which in turn gave rise to the white-skinned berry mutant, and ii) the parallel model, where the black-skinned berry ancestor gave rise to the grey-skinned and the white-skinned berry mutants separately. Here, we propose the parallel model as the evolutionary model for the formation of Pinot berry colour somatic variants. According to this novel model, the somatic mutants Pinot gris and Pinot blanc arose from the ancestral Pinot noir cultivar independently. We name this parallel model as the "Pinot-model", distinctly from the previously reported sequential "CabSau-model". Moreover, these results elucidated the relationship between Pinot blanc and Pinot gris. Finally, we suggest the name Pinot verdâtre for the unpigmented bud sport of Pinot gris, holding a peculiar genetic make-up and a green-like phenotype. Our findings represent a breakthrough towards the full understanding of the mechanisms behind the formation of white, grey, red, and pink grape cultivars, the overall phenotype of which determines a specific enological aptitude.

Riassunto in italiano

TORNA ALL'ELENCO – BACK TO THE TOP

Pinot bianco e Pinot grigio sono mutazioni somatiche indipendenti di Pinot nero

In vite l'accumulo e la stabilizzazione delle mutazioni somatiche sono eventi frequenti, che permettono ai viticoltori di selezionare e propagare nuove cultivar. Molte delle mutazioni somatiche non influiscono sull'intero meristema, ma solo su una sua porzione (chimere). Come conseguenza di questa struttura meristematica a strati, le chimere sono costituite da due layer (foglietti embrionali) adiacenti, ma geneticamente distinti. In vite, tra le mutazioni somatiche spontanee, sicuramente quella relativa al colore della bacca è la più conosciuta. Il colore della bacca è determinato da un'unica famiglia di pigmenti, gli antociani, che variano enormemente sia in concentrazione che in composizione a seconda della cultivar. In molte piante la biosintesi degli antociani è controllata da geni regolatori appartenenti a fattori di trascrizione della famiglia MYB; in particolare in Vitis vinifera L. i maggiori regolatori sono VvMybA1 e VvMybA2. L'inserzione del retrotrasposone Gret1 nel promotore di VvMybA1 e uno SNP non sinonimo nella regione codificante di VvMybA2 danno origine al fenotipo a bacca bianca. Recentemente diverse linee di ricerca, sia a livello genetico che genomico, hanno dimostrato che il locus del colore è composto da un gruppo di quattro geni Myb-like localizzati sul cromosoma 2. Il Pinot, essendo una varietà diffusa in tutto il mondo, nonchè una delle fondatrici, ha avuto molteplici possibilità di subire delle mutazioni somatiche, molte delle quali hanno modificato il colore originario della bacca dando origine a Pinot blanc e Pinot gris. Nonostante la relazione tra Pinot blanc e Pinot gris non sia stata ancora chiaramente delineata, secondo il modello evolutivo più accreditato il Pinot blanc deriva dal Pinot gris che, a sua volta, deriva dal Pinot noir. Il Pinot gris è sicuramente una chimera periclinale di Pinot noir, ma la natura precisa di questa modificazione genetica resta da determinare.

Il nostro studio ha indagato questo modello evolutivo. Basandoci sull'analisi strutturale del locus del colore a livello layer-specifico in 4 cloni di Pinot noir, 10 di Pinot blanc e 13 di Pinot gris, insieme ai suoi mutanti spontanei, abbiamo dato una spiegazione evoluzionistica delle mutazioni somatiche che hanno modificato il locus del colore. Mediante lo studio della dinamica strutturale lungo il cromosoma 2, è stata identificata e caratterizzata una delezione molto ampia (circa 4.300 Kb) in un singolo layer di Pinot gris, mentre è stata individuata una delezione corta (circa 150 Kb) in entrambi i layer di Pinot blanc. Nell'ambito della medesima cultivar, tutti i cloni hanno mostrato lo stesso profilo (lunghezza) di delezione. Come osservato recentemente in altri cloni di Pinot noir, possiamo supporre che i cambiamenti strutturali che si sono verificati in Pinot gris e Pinot blanc siano stati mediati da un fattore di stress che ha provocato l'attivazione di un elemento genetico mobile.

Teoricamente, l'origine di un mutante a bacca meno colorata può essere attribuito a due modelli distinti: 1) il modello sequenziale, in base al quale un ancestrale a bacca nera ha dato origine a un mutante a bacca grigia che, a sua volta, ha dato origine a un mutante a bacca bianca; 2) il modello parallelo, in base al quale un ancestrale a bacca nera ha dato origine a un mutante a bacca grigia e a un mutante a bacca bianca in maniera indipendente. Mediante questo studio proponiamo un modello parallelo come modello evolutivo alla base della formazione delle varianti somatiche per il colore in Pinot. In base a questo modello innovativo, i mutanti somatici Pinot gris e Pinot blanc si sono originati indipendentemente dalla cultivar ancestrale Pinot noir. Diversamente dal "modello Cab-Sau" sequenziale, precedentemente riportato in letteratura, ci riferiamo a questo modello parallelo come al "modello-Pinot". Questi risultati hanno inoltre delucidato la relazione tra Pinot blanc e Pinot gris. Suggeriamo infine il nome Pinot verdâtre per la mutazione di Pinot gris a bacca non colorata, dal momento che presenta un peculiare assetto genetico e un colore della bacca verdognolo. Le nostre scoperte rappresentano un passo in avanti verso la comprensione completa dei meccanismi che sono alla base della formazione di cultivar a bacca bianca, grigia, rossa e rosa, il cui fenotipo complessivo è responsabile di una specifica attitudine enologica.

9 – Dismissing enological myths: effect of ethanol and maceration enzymes on grape seed tannin extraction. Enological implications

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During maceration, proanthocyanidins are extracted from skin and seeds. It is commonly accepted that skin proanthocyanidins are more readily extractible, whereas extraction from seeds requires longer maceration and is favored by the presence of ethanol.
Wine proanthocyanidin composition can be manipulated by winemaking practices, with several of these practices based on the assumption that ethanol from fermentation is necessary to disorganize the outer lipidic layer that covers and isolates the seeds, meaning that seed proanthocyanidins are only extracted if ethanol is present. In this way, low temperature prefermentative macerations are designed to increase the extraction and stabilization of the polyphenolic compounds (anthocyanins and proanthocyanidins) from skins and it is commonly assumed that the extraction of the more aggressive seed proanthocyanidins would be very limited since alcohol is not present
Trying to clarify this, we used a model solution to determine how ethanol and time affect the amount and characteristics of proanthocyanidins extracted from seeds. Spectrophotometric and chromatographic results showed that ethanol was not required for proanthocyanidin extraction, although its presence increased the rate of extraction and that macerating the seeds in a solution containing just water prepares the seeds for a very fast extraction of tannins when ethanol is present. These findings suggest that also the length of extraction time is an important consideration when managing techniques such as cold soak which are thought not to affect seed proanthocyanidin extraction.
Also, maceration enzymes have long been used to improve the extraction of phenolic compounds from skins, but their role in seed degradation and the release of seed phenolic compounds has not been taken into account. We have also described the effect of different pure enzyme activities (xylanase, cellulase, polygalacturonase and pectinmethylesterase) and commercial preparations on the release of proanthocyanidins from grape seeds. The results demonstrate that some enzymatic activities, especially polygalacturonase and cellulase, favour the degradation of seed cell walls, promoting the diffusion of seed proanthocyanidins.

Resúmen en castillano

Destruyendo mitos enológicos: efecto del etanol y las enzimas de maceración en la extracción de taninos de semillas. Implicaciones enológicas
Durante la maceración, los taninos se extraen de las pieles y las semillas al vino. Se acepta comúnmente que los taninos de las pieles son más fácilmente extraíbles que los de semilla, que requieren tiempos de maceración más largos y la presencia de etanol.
La composición en taninos de un vino se puede intentar manipular con diferentes prácticas enológicas, basándose muchas de esas prácticas en asumir que el etanol de la fermentación es necesario para desorganizar la capa lipidica externa que cubre y aísla la semilla, y por tanto, que los taninos de semilla solo se extraerán cuando el etanol esté presente. Por ello, técnicas como la maceración prefermentativa en frio se diseñan para favorecer, en ausencia de etanol, la extracción de compuestos fenólicos de las pieles, y no los de la semilla, ya que el etanol no está presente.
Pero ya que estos hechos no han sido científicamente comprobados, hemos usado una disolución modelo para determinar como el etanol y el tiempo de maceración afectan la cantidad y composición de los taninos

extraídos de las semillas. Los resultados espectrofotométricos y cromatográficos mostraron que el etanol no era necesario para la extracción de taninos, aunque su presencia incrementa el ritmo de extracción. También mostraron que la maceración de semillas en un medio acuoso, sin la presencia de etanol, "prepara" la semilla para una extracción más rápida de los taninos cuando el etanol esté presente. Esto es muy importante en técnicas como la maceración prefermentativa en frio, que podría preparar a las semillas para una importante extracción de sus taninos durante la fase de maceración fermentativa, cuando el etanol esté presente.
También, las enzimas de maceración se han utilizado durante mucho tiempo para facilitar la extraccion de compuestos fenólicos de las pieles pero su papel en la degradación de semillas no ha sido estudiado. Hemos investigado el efecto de enzimas puras (xilanasa, celulasa, poligalacturonasa y pectinmetilesterasa) y preparaciones enzimáticas comerciales en la liberación de taninos de semillas. Los resultados demuestran que algunas actividades enzimáticas, especialmente poligalacturonasa y celulasa, favorecen la degradación de las paredes celulares de las semillas, promoviendo la difusión de los taninos de semilla, lo cual debe ser tenido en cuenta al utilizar enzimas de maceración durante la vinificación.

10 – Improving Grape Phenolic Content and Wine Chromatic Characteristics through the Use of Elicitors

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In winegrapes, the technological importance of phenolic compounds, especially flavonoids, is well known. They are responsible for the color of wines, especially anthocyanins (colored pigments responsible for the chromatic characteristics of red wines), proanthocyanidins (responsible for the long term stability of red wine color) and flavonols (compounds that may influence wine color through copigmentation), and some other organoleptic properties such as astringency, bitterness and body. Another important aspect that has been widely studied in recent years is the role of grape and wine phenolic compounds in the human diet and health.
Several approaches have been proposed for improving the phenolic content of grapes. Among them, the most recent approach is the use of elicitors. In plants, phenolic compounds contribute significantly to plant resistance against pests, pathogens and environmental stress and their concentration in plant tissues may increase markedly as part of this resistance phenomenon. However, it has been demonstrated that the accumulation of phenolic compounds can also be induced, or enhanced by the exogenous application of natural or synthetic compounds that mimic the signalling molecules that trigger all the resistence phenomena.
Two examples of such compounds are benzothiadiazole (BTH) and methyl jasmonate (MeJ). The objective of our study has been to test, during three years, if the preharvest treatment of vines with BTH, MeJ and a mixture of BTH and MeJ affected the accumulation of the main flavonoid compounds (anthocyanins, flavonols and flavanols) in grapes and in their resulting wines, looking forward to obtaining wines with an improved color and organoleptical characteristics.
The results indicated that the anthocyanin, flavonol and proanthocyanidin content of grapes could be improved with these treatments. Moreover, the wines obtained from the treated grapes showed higher color intensity and total phenolic content than the wines made from control grapes. Therefore, the exogenous application of these elicitors, as complement to fungicide treatments, could be an interesting strategy for increasing the phenolic content of the grapes and the resulting wines, improving, at the same time, vine protection against pathogens.

Resúmen en castillano

Mejora del contenido fenólico y de las características cromáticas en uvas y vinos utilizando elicitores
Es bien conocida la importancia que los compuestos fenólicos tienen en las uvas para vinificación. Son responsables del color de los vinos, especialmente los antocianos (los pigmentos coloreados responsables de las características cromáticas de los vinos), las proantocianidinas o taninos (responsables de la estabilidad del color del vino en el tiempo) y los flavonoles (compuestos que pueden influir sobre el color del vino a través de la copigmentación), y otras propiedades organolépticas como la astringencia, amargor, cuerpo. Otro aspecto importante que ha sido ampliamente estudiado en los últimos años es el papel de los compuestos fenólicos de la uva y el vino en la dieta humana y la salud.
Varios mecanismos se han propuesto para mejorar el contenido fenólico de las uvas. Entre ellos, el más reciente es el uso de elicitores. En las plantas, los compuestos fenólicos contribuyen de una forma significativa a la resistencia contra plagas y estrés medioambiental y su concentración

en los tejidos vegetales se puede incrementar de forma notable como parte de este fenómeno de resistencia. De todas formas, se ha demostrado que la acumulación de compuestos fenólicos también puede ser inducida o incrementada por la aplicación de compuestos naturales o sintéticos que imitan a las moléculas señal que disparan los fenómenos de resistencia.
Dos ejemplos de estos compuestos son el benzotiadiazol (BTH) y el metil jasmonato (MeJ). El objetivo de nuestro estudio ha sido probar, durante tres años, si el tratamiento precosecha de uvas de la variedad Monastrell con BTH, MeJ y una mezcla de ambos elicitores afecta a la acumulación de los principales compuestos flavonoides (antocianos, flavonoles y flavanoles) en uvas y en sus vinos, buscando obtener vinos con unas características cromáticas y organolépticas mejoradas.
Los resultados indican que el contenido en compuestos flavonoides puede ser incrementado con estos tratamientos. Además, los vinos de esas uvas tienen mayor intensidad de color y compuestos fenólicos totales. Por tanto, la aplicación de estos compuestos, que además se pueden utilizar como complemento a un tratamiento fungicida, podría ser una práctica interesante para incrementar el contenido fenólico en uvas, mejorando, al mismo tiempo, la protección de la viña contra patógenos.

26 – An effective solution to limit biogenic amines in winemaking: Enzymatic extracts from vineyard fungi able to reduce biogenic amines content in white and red wines

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Currently, on the market there are no definitive procedures for the control of biogenic amines in wine. The main strategy followed by wineries is based on prevention, to avoid the formation of these potentially toxic compounds. Following a novel approach, this study makes available, to the enological sector, a natural treatment that reduces the concentration of biogenic amines in wines. The procedure is based on the employment of enzymatic extracts from fungi isolated from the grapevine, this is to say, from natural ecological niches closely related to the aim sought for application, and so far unexplored for such purposes.
For the induction of enzymatic activities, the fungal isolates of the grapevine were incubated in minimal growth medium supplemented with biogenic amines (histamine, tyramine or putrescine) as sole nitrogen source. From a preliminary screening of 40 fungal strains active on the degradation of histamine, tyramine and/or putrescine, it was selected the strain of *Penicillium citrinum* CIAL-274, 760, that exhibited a percentage of degradation of the three amines close to 100%, in culture medium. The enzymatic extracts of this fungus have been tested in white and red wines containing higher concentrations of biogenic amines, resulting in an effective decrease of their content.

Resúmen en castillano

Una solucion efectiva contra las aminas biógenas en enologia: extractos enzimáticos de hongos de la vid que reducen el contenido de aminas biógenas en vinos blancos y tintos
En la actualidad, en el mercado no existen procedimientos definitivos para el control de aminas biógenas en el vino. La principal estrategia que siguen las bodegas está basada en la prevención para evitar la formación de estos compuestos potencialmente tóxicos. Con un enfoque novedoso, este trabajo plantea la puesta a disposición del sector enológico de un tratamiento de origen natural que permite reducir la concentración de aminas biógenas en vinos. El procedimiento se basa en el empleo de extractos enzimáticos de hongos aislados de la planta de la vid, es decir, de nichos ecológicos naturales e íntimamente relacionados con el fin que se persigue en la aplicación, y hasta ahora inexplorados para tales fines.
Para la inducción de la actividad enzimática, los hongos aislados de la vid se incubaron en medios mínimos de crecimiento suplementados con aminas biógenas (histamina, tiramina o putrescina) como única fuente de nitrógeno. A partir de un screening preliminar de 40 cepas fúngicas activas en la degradación de histamina, tiramina y/o putrescina, seleccionamos la cepa de *Penicillium citrinum* CIAL-274, 760, con un porcentaje de degradación de las tres aminas cercano al 100 %, en medios de cultivo. Los extractos enzimáticos de este hongo se han probado en vinos blancos y tintos conteniendo elevadas concentraciones de aminas biógenas, consiguiéndose una disminución efectiva del contenido de las mismas.

TORNA ALL'ELENCO – BACK TO THE TOP

31 – Recovery of aromatic aglycones from winemaking by-products

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Waste grape skins from the wine industry are an abundant byproduct, currently underexploited. The lack of relevant industrial applications results in accumulation, causing contamination and significant economical problems to producers. However, grape skin is an important source of odorless glycosidic aroma precursors that under hydrolysis might release aroma compounds. So, grape pomace can be considered an interesting source to obtain natural aroma compounds with potencial applications in different industrial sectors (agro-food, cosmetic, perfumery, etc).
The objective of this work has been firstly to check the potencial of grape pomace as a source of glycosides, and secondly, to know the feasibility of green extraction technologies (such as, Pressurized liquid extraction (PLE) and Supercritical fluid extraction (SFE)) for the extraction of theses glycosides comparing it with the more conventional liquid-liquid extraction (LLE).
The results of this work show that grape pomace by-products can be a source of glycosidic aroma precursors that can release interesting odorant compounds (monoterpenes, norisoprenoids, benzenoids compounds,e tc.) characterized by low aroma thresholds and interesting sensory properties. The use of PLE, SFE greatly improves the extraction compared to the more conventional LLE. For example, limonene shows concentrations of 0.9, 4.3 and 11.5 µg/kg by the use of LLE, PLE and SFE, respectively. This work show an interesting alternative for the recovery and valorization of grape by-products and besides, reducing their environmental consequences.

Resúmen en castillano

Recuperación de aromas varietales a partir de subproductos generados durante la elaboración del vino
Los hollejos de uva son uno de los subproductos más abundantes de la industria vitivinícola. Actualmente la ausencia de aplicaciones de carácter industrial para aprovechar estos residuos, provoca su acumulación, lo cual deriva en una contaminación medioambiental e importantes costes económicos para los productores. Sin embargo, las pieles de uva son un importante reservorio de precursores del aroma glicosilados no odorantes que mediante hidrólisis pueden liberar las correspondientes agliconas odorantes. Por tanto, estos residuos sólidos, en forma de orujos, pueden considerarse una posible fuente para la obtención de aromas naturales con potenciales aplicaciones en diferentes sectores industriales (cosmético, fragancias, alimentación).
En este estudio se ha evaluado el potencial de estos subproductos como fuente de obtención de precursores del aroma, comparándose distintas metodologías limpias de extracción (i.e. extracción con líquidos presurizados (PLE) y la extracción con CO2 supercrítico (SFE)) con el método de extracción convencional líquido-líquido (LLE).
Los resultados indican que los orujos de uva constituyen una fuente de precursores glicosilados de aroma que pueden generar una gran cantidad de compuestos odorantes (monoterpenos, norisoprenoides, compuestos bencénicos) caracterizados por presentar bajos umbrales de percepción, y en general, notas aromáticas positivas. Además, se ha comprobado que el empleo de técnicas como PLE y SFE supone un mayor rendimiento de extracción comparado con el empleo de la más convencional LLE. Por ejemplo, para el limoneno se obtuvieron concentraciones de 0.9, 4.3 y 11.5 µg/kg utilizando LLE, PLE y SFE, respectivamente. Por tanto, este trabajo abre una interesante vía de revalorización de este excedente agrícola permitiendo además la disminución del impacto ambiental de estos residuos.

35 – Comparative evolution of dissolved gases and SO_2 during storage of a rosé wine of Cinsault bottled in PET and glass bottles

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Oxygen is one of the main factors for wine's oxidation and carbonic gas is a major support of the sensory quality of wines. At bottling, CO_2 for white and rosé wine must be adjusted to a wanted level between 800 and 1200 mg/L, whereas O_2 must be reduced as much as possible. Transfer of oxygen through the packaging material (bottle and stopper) after bottling is regulated by containers OTR. Both management of dissolved gases during bottling and choice of package are the key factors to control wine quality and enable packers and distributors to act on the wine's shelf life.
To respect these standards, the R&D collaborative project aims to develop, after 3 years, an industrial answer which will be an alternative to glass bottles for quality rosé wines, by using lightened, recycled monolayer PET with oxygen scavengers. The first step consisted in assessing limits of lightened monolayer virgin or recycled PET without oxygen scavenger. So a first experimentation on a rosé wine of Cinsault was made at the Experimental unit of Pech-Rouge (INRA, Gruissan). Three kinds of 75cl clear PET bottles (34g, 38g, 38gR (100% recycled)) were compared to clear glass bottle. Total oxygen content (TO_2) was equal to 6.2 ± 0.5 mg/btl including 78 ± 1.7% trapped in headspace and total carbon dioxide content (TCO_2) was 602 ± 17 mg/btl. Bottles were closed with screw caps (Novatwist with Saranex seal) and stored upright at 20 ± 0.7 °C, with a relative humidity of 67.6 ± 9.1 %. Half was stored in the light and the other half in the dark (cardboard boxes). Analytical monitoring (aphrometric pressure, headspace volume, O_2, CO_2 and SO_2) was carried out for 372 days.
Analysis show a most important evolution for wine ageing in PET bottles compare to ageing in glass bottles. Nevertheless, no difference between PET 34g and even between PET 38g and PET 38gR is brought to light.
Aphrometric pressure of PET bottles decreases gradually from -18 to -68 hPa in 372 days, and the TCO_2 content decreases of about 40% in one year, whereas slight fluctuations of these parameters are observed for glass bottles.
After consumption of O2 trapped during bottling, TO_2 content in glass bottles remains stable: 0.1 mg/btl from the 77th to the 372th day, and for PET bottles: 0.4 mg/btl until the 6th month. Then, between the 6th and the 12th month, gaseous and dissolved O_2 increase gradually to reach a TO_2 content between 1.2 and 1.7 mg/btl after one year.
Free SO_2 concentration for wine in PET bottles reaches the critical threshold of 10 mg/L after 3 months. It is admitted that under this threshold wine is no longer protected against oxidation. Comparatively, wine in glass bottles has still 20 mg/L after one year.
From the 77th day, important decrease of CO_2 and SO_2 and higher TO_2 content for wine in PET bottles are bound to a high gases permeability of monolayer PET without oxygen scavenger.
PET 34g is different from PET 38g and 38gR only by losses 4% higher of CO_2 and by a higher increase of TO_2 content at 6 month. The use of 100% recycled PET has no effect on wine conservation compare to virgin PET.
Light noticeably accelerates TO_2 consumption. Visible consumption of 90% of TO_2 trapped during bottling is reached between the 30th and 45th day in the light. In the dark 30 days more are necessary. Light effect is more difficult to observe on other parameters.
Finally, kinetic monitoring of partial pressures in gas and liquid phases highlights opposing behaviors of O_2 and CO_2 in a bottle.
A second bottling with recycled PET and oxygen scavengers associated with a stronger management of dissolved gasses during bottling is being carried out. The aim of this study is to be able to transfer an eco-responsible alternative to glass bottle in an industrial process, whereas a

shelf-life of 9 months must be guaranteed, which fits with the usual distribution network of the studied wine.

Resumé en français

Évolution comparative des gaz dissous et du SO_2 lors de la conservation d'un vin rosé de Cinsault en bouteille PET par rapport au verre

L'oxygène est le facteur majeur de l'oxydation des vins et le gaz carbonique un support majeur de la qualité sensorielle des vins. À l'embouteillage des vins blancs et rosés, le CO_2 doit être ajusté à un niveau désiré compris entre 800 à 1200 mg/L et l'O_2 doit être réduit du mieux possible, les entrées d'O_2 post-conditionnement étant régulées par l'OTR du contenant (obturateur et bouteille). La gestion des gaz dissous au conditionnement et par l'emballage sont les 2 piliers maîtrisables par le metteur en marché lui permettant d'agir sur la DLUO du vin.

Dans le respect de ces exigences, le projet collaboratif R&D a pour ambition de mettre au point, au bout de 3 ans, une solution industrielle alternative au verre par l'utilisation de PET monocouche allégé et recyclé avec absorbeur d'oxygène, pour des vins rosés de qualité. La première étape a consisté à évaluer les limites du PET monocouche allégé sans absorbeur, vierge ou recyclé.

Une 1ère mise en bouteille expérimentale a donc été effectuée à l'unité expérimentale INRA de Pech-Rouge sur un vin rosé de Cinsault. 3 types de bouteilles PET de 75 cL (34g, 38g, 38gR [100% recyclé]) ont été comparés à la bouteille verre. La teneur en O_2 total était de 6,2 ± 0,5 mg/bt dont 78 ± 1,7% d'O_2 piégé dans l'espace de tête et une teneur en CO_2 total de 602 ± 17 mg/bt. Les bouteilles ont été serties avec des capsules Novatwist avec joint Saranex, puis conservées debout à 20 ± 0,7 °C avec une humidité relative de 67,6 ± 9,1 % moitié à la lumière, moitié à l'obscurité (en cartons). Un suivi analytique (pression aphrométrique, volume dégarni, O_2, CO_2 et SO_2) a été effectué sur une durée de 372 j.

Les analyses font ressortir l'évolution plus importante des vins conservés en bouteilles PET par rapport à ceux conservés en bouteilles verre, sans toutefois mettre en évidence une différence entre les PET 34g, et surtout entre les PET 38g et 38gR.

En 372 jours, sur les bouteilles en PET la pression aphrométrique diminue progressivement de -18 à -68 hPa et la teneur en CO_2 total diminue d'environ 40 % en 1 an, alors que ces 2 paramètres varient peu pour le verre.

Après la phase de consommation de l'O_2 piégé au conditionnement, la teneur en O_2 total se stabilise à 0,1 mg/bt du 77ème au 372ème j dans les bouteilles en verre. Pour les bouteilles en PET, la teneur reste stable à un niveau de 0,4 mg/bt jusqu'au 6ème mois. Ensuite, entre le 6ème et le 12ème mois, les teneurs en O_2 gazeux et dissous remontent progressivement jusqu'à atteindre au bout d'1 an, une teneur en O_2 total se situant entre 1,2 et 1,7 mg/bt.

La concentration en SO_2 libre des vins conservés en PET atteint au bout de 3 mois le seuil critique des 10 mg/L à partir duquel le vin n'est plus communément jugé protégé de l'oxydation, tandis que les vins en bouteille verre contiennent encore 20 mg/L au bout d'1 an.

Les fortes diminutions en CO_2, SO_2 et les teneurs plus élevées à partir du 77ème j en O_2 total des vins conservés en PET sont à relier avec une forte perméabilité aux gaz du PET monocouche sans absorbeur d'oxygène.

Le PET 34g se différencie seulement des PET 38g et 38gR par des pertes en CO_2 supérieures de 4% et par une remontée supérieure de l'O_2 total à 6 mois. L'utilisation du PET 38g 100% recyclé n'a pas eu d'impact sur la conservation des vins vis-à-vis du PET vierge.

La lumière accélère sensiblement la consommation d'oxygène total. La consommation apparente de 90% de l'O_2 total piégé au conditionnement est atteinte entre le 30ème et le 45ème j à la lumière. À l'obscurité, il faut attendre 30 j supplémentaires. L'impact de la lumière est plus difficile à mettre à évidence sur les autres paramètres.

Enfin, le suivi des cinétiques des pressions partielles des phases gazeuse et liquide met en évidence les comportements opposés de l'O_2 et du CO_2 dans une bouteille.

Un deuxième conditionnement mettant en œuvre du PET recyclé intégrant des absorbeurs d'oxygène et une plus grande maîtrise des gaz dissous à l'embouteillage est en cours de suivi. Son objectif est de pouvoir transférer industriellement une alternative éco-responsable à la bouteille en verre tout en assurant une DLUO de 9 mois correspondant au circuit commercial du vin étudié.

38 – Full automation and control of vinifications by FT-NIR spectroscopy: An innovation presenting ground-breaking opportunities

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In the last decade, a variety of process analysis devices have been introduced which allow winemakers to monitor alcoholic fermentations. These systems visualize the fermentation rates and progress, but are too inaccurate to estimate actual sugar concentrations during fermentations. We present an innovative FT-NIR spectroscopy based system that provides accurate real-time information about key-fermentation parameters including glucose, fructose, and ethanol concentrations throughout fermentations. Integrating this in-line sugar analysis with process control enables fully automatic fermentations and new ground-breaking strategies. Specifically, the system allows to process high sugar containing musts (e.g. for the production of hot climate, late harvest or icewines) without causing the yeast hyperosmotic stress response, which leads to increased formation of undesirable byproducts such as acetic acid and acetaldehyde, and, possibly, fermentation problems. This can be achieved with a continuous fed-batch fermentation where must is slowly and automatically added to the fermentation tank at such rates as to keep sugar concentrations constant at low levels (e.g. 50 g/l) during the fermentation. We will present data that shows that such an approach leads to a 80 90% reduction in acetic acid values and also decreases acetaldehyde levels by 50%. In addition, cooling costs can be reduced by 1/3 since the cold settled must partially cools the fermentation during additions, and smaller yeast inocula can be used. Some examples of white and red wine fermentations will be presented and the effect of this innovative fermentation management strategy on sugar, acid and ester composition, yeast viability, and sensory aspects will be discussed, as well as its application to malolactic fermentation. An animated visualization of this “substratostat” system is currently provided at http://www.youtube.com/watch?v=kEUvYFA0lj0

39 – Assessment of oxidation during winemaking technological processes: a new approach based on the measurement of phenolic compounds in musts

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Once the break of the cellular grape berry compartimentation occurred during the various technological operations (de-stemming, pressing, crushing, etc.), the dissolution of the oxygen in the must leads to a certain must. This cellular disintegration indeed puts in contact the substrates of oxidation - the native phenolic compounds of the grape -, oxygen and the enzymatic polyphenoloxidase activity (PPO) of the grape. The colour of the must then modifies, evolving towards brown tones more or less pronounced with frequently a change of its transparency and its aroma.
In rather sensitive or very sensitive musts to oxidation, a regeneration of the initial phenolic substrates occurred by a reaction of coupled oxidation, immediately followed by a new oxidation being translated by a greater consumption of oxygen. The capacity of a must to oxidise can be thus measured only by measuring finely the consumption of oxygen of this must in very controlled conditions, but also by measuring the evolution of hydroxycinnamic acids, the GRP (Grape Reaction Product) and their quinones during this oxygen consumption. It is through this methodology that we were able to measure finely the degree of oxidation of the must throughout the various technological stages of its elaboration.
We were thus able to finely quantify the level of oxidation of several white musts occurring during mechanical harvest and transport to the wineries, or during all the steps of pressing either through classical pneumatic press or through a centrifuge decanter.
Contrary to what is generally admitted, the level of must oxidation is very similar with both types of pressing systems, even if the duration of the grape pressing is very different according to the technology used. Fast pressing systems such as centrifuge decanter technology could be therefore easily used for continuous processing of white musts in liquid phase.
Moreover, the effect of lowering harvest temperature from 20°C to about 5°C was quantified all along the must processing chain with respect to must oxidation.
The protective effect against the oxidation of a reduction in the temperature of the grape harvest is not necessarily the most important during the stages of harvest and transport of the grape harvest towards the cellar, but is especially important during the operations of pressing, with a stronger efficiency on juices obtained at the end of press than on the pressing first ones. This observation gives in fact several new informations onto the protection against the oxidation during the pressing: a) the complete inerting of a press such as developed by certain manufacturers can be advantageously replaced by a reduction in the temperature of the grape harvest and its preservation during the pressing, b) the valuation of the end juices of press can also be reached by a specific cooling of the grape harvest with a very strong qualitative gain.
It is recognized that maintaining the harvested grapes at a relatively low temperature until the fermentation is a quality factor for white and rosé wine making. The benefits of this practice are generally attributed to a slower diffusion of compounds of the skins to the must, but also to a limitation of oxidation, a facilitation of settling and a limitation of inappropriate departures in spontaneous fermentation. During the 2011 harvest, we have finely measured by a new technology the oxidation of the must all throughout the different technological steps of grape processing in order to better understand the real impact of lowering temperature until fermentation. It appears that maintaining the harvest at a low temperature coupled with a few simple precautions can lead to no detectable oxidation in the grapes until the pressing step. During pressing, the oxidation can also be greatly slowed by lowering the temperature.

Resumé en français

Mesure de l'oxydation pendant divers process technologiques en oenologie: une nouvelle méthodologie basée sur la mesure des composés phénoliques dans les moûts

Lorsqu'il y a rupture de la compartimentation cellulaire végétale dans la baie de raisin au cours de divers traitements technologiques (récolte, foulage, pressurage, etc.), la dissolution de l'oxygène dans le moût conduit à un certain nombre de réactions d'oxydation qui peuvent modifier la composition chimique du moût. Ceci est dû à la mise en contact intime des substrats des réactions d'oxydation – les composés polyphéniques natifs du raisin - , de l'oxygène et de l'activité enzymatique polyphénoloxydase du raisin. Au cours de ces réactions, la couleur du moût se modifie, et évolue vers des teintes brunâtres plus ou moins prononcées, accompagnant souvent une altération de la transparence et des arômes de celui-ci.

Dans les moûts de raisin sensibles à l'oxydation, au cours des premières étapes des réactions d'oxydation, on observe la régénération des substrats phéniques natifs du raisin par réaction d'oxydation couplée, immédiatement suivie par de nouvelles réactions d'oxydation impliquant une consommation accrue d'oxygène. La capacité d'un moût de raisin à l'oxydation peut donc être seulement mesurée soit par une mesure précise de la consommation d'oxygène en conditions extrêmement contrôlées, soit par mesure de l'évolution des acides hydoxycinnamiques, du GRP (Grape Reaction Product) et de leurs quinones correspondantes au cours de l'oxydation. C'est en se basant sur cette seconde méthodologie que nous avons pu finement mesurer le degré réel d'oxydation de moûts de raisin au cours de différentes étapes technologiques de leur élaboration.

Ainsi, nous avons pu finement quantifier le niveau d'oxydation de divers moûts blancs durant la récolte mécanique et le transport à la cave, mais également durant les diverses étapes de pressurage soit au travers d'un pressoir pneumatique classique, soit au travers de l'utilisation d'un décanteur centrifuge.

Contrairement à ce qui pourrait être admis, les niveaux d'oxydation atteint par les moûts est trés similaire avec les deux types de technologies utilisées, alors que les temps de résilience dans les deux types d'appareillage est très différent. Les systèmes rapides de pressurage tels que le décanteur centrifuge peuvent donc être aisément utilisés pour des process continus d'obtention de moûts blancs en hase liquide à partir de vendange entière.

De plus, l'effet d'un refroidissement de la vendange de 20°C à 5°C à la récolte et au cours de l'élaboration du moût a été quantifié quant à son effet potentiel sur le niveau d'oxydation des moûts à toutes les étapes technologiques de son obtention.

Cet effet protecteur d'un abaissement de la température envers les phénomènes d'oxydation des moûts n'est pas le plus important pendant les étapes de récolte ou de transport de la vendange. C'est essentiellement durant les phases de pressurage que cet effet est sensible, et surtout sur les dernières pressées. Cette dernière observation donnent en fait de nouvelles pistes concernant la protection contre l'oxydation au cours du pressurage : a) L'inertage complet d'un pressoir, tel que développé par certains fabricants pourrait être avantageusement remplacé par une réduction de la température de la récolte, et son maintien à basse température pendant les étapes de pressurage, b) La mise en valeur des dernières presses pourrait être obtenue grâce à un refroidissement spécifique de la vendange avec un très fort gain qualitatif.

40 – Electrodialysis, a physical and multipurpose process for an enology of precision: last development, pH adjustment of wines by bipolar membranes

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Electrodialysis is a membrane electro-process. Its principle consists in extracting ions through a stack of non-porous ionic membranes under the effect of an electric potential difference. According to the type of membranes used (anionic, cationic, bipolar) and their organization into the stack, electrodialysis is a multipurpose process offering various treatments possibilities. The research studies carried out by Experimental Unit INRA of Pech-Rouge, in partnership with the Eurodia/Oenodia company, demonstrated the potentialities of electrodialysis and its interest for the wines treatment.
The first application developed in the winemaking was the tartaric stabilization treatment of the wines before bottling. The use of a conventional stack alternating anionic and cationic membranes, makes it possible to extract in a combined way, the necessary and sufficient proportion of potassium and hydrogenotartrate from the wine, to reach tartaric stability. This application admitted by the OIV in 1995 allowed the electrodialysis integration in enology. This technology appears in the list of the authorized practices of the European regulatory texts since January 2000.
To answer the problems of the wines evolution, consecutive to the climate changes (rise of pH), the second studied application, then developed, concerned the controlled acidification of the wines. The carried out studies demonstrated that the association of bipolar and cationic membranes ensures an exclusive cations extraction, essentially potassium, during the treatment. This selective extraction allows to reduce the wines pH and to correct initial imbalance. The wines acidification by electrodialysis was admitted by the OIV in 2010 and is now authorized in Europe since 2011.
Recently, the works realized on electrodialysis concerned its capacities of wines deacidification (increase of the pH). Indeed, certain wine-producing areas cultivate for climatic reasons specific vines varieties which produce strongly acid musts and consequently produce wines with very low pH. These low pH values are due to the strong concentrations in organic acids. So, studies were carried out on french white and red wines and were supplemented by experiments on a wine from Quebec. They highlighted on the laboratory scale, the selective extraction of these acids thanks to a stack which associates anionic and bipolar membranes, and thus increasing the pH. Thus, the electrodialysis interest for the wines deacidification was demonstrated and then adding a new function to the process. This application was admitted in 2012 by the OIV.
Thus, the electrodialysis process allows, with the same technological device, equipped with various configuration membranes stack and adapted sensors (conductimeter, pH-meter) to ensure the controlled treatments of tartaric stabilization, and henceforth of pH adjustment. Controllable, the developped equipment makes it possible to very precisely reach the target values for a perfectly controlled wines quality, without the use of additive.

Resumé en français

L'électrodialyse, un procédé physique polyvalent pour une œnologie de précision: dernier développement, l'ajustement du pH des vins par membranes bipolaires

L'électrodialyse est un procédé électro-membranaire. Son principe consiste à extraire des ions au travers d'un empilement de membranes ioniques denses sous l'action d'une différence de potentiel électrique. Selon le type de membranes utilisées (anionique, cationique, bipolaire) et leur organisation au sein de l'empilement, l'électrodialyse est polyvalente offrant diverses possibilités de traitements. Les travaux de recherche conduits par l'Unité Expérimentale INRA de Pech-Rouge, en partenariat avec la société Eurodia/Oenodia, ont démontré les potentialités de l'électrodialyse et son intérêt pour le traitement des vins.
La première application développée dans la filière a été le traitement de stabilisation tartrique des vins avant conditionnement. L'utilisation d'un empilement conventionnel alternant membranes anioniques et membranes cationiques, permet d'extraire de façon combinée, la proportion nécessaire et suffisante de potassium et d' hydrogénotartrate du vin, pour atteindre la stabilité tartrique. Cette application admise par

l'OIV en 1995 a permis l'intégration de l'électrodialyse dans la filière.. Cette technologie figure dans la liste des pratiques autorisées du règlement européen depuis janvier 2000.
Pour répondre à la problématique de l'évolution des vins, consécutive aux changements climatiques (hausse de pH), la seconde application étudiée, puis développée, a concerné l'acidification maîtrisée des vins. Les travaux réalisés ont démontré que l'association de membranes bipolaires et cationiques permet d'assurer une extraction exclusive des cations, essentiellement du potassium, lors du traitement. Cette extraction sélective permet de réduire le pH des vins et de corriger ainsi le déséquilibre initial. L'acidification des vins par électrodialyse a été admise par l'OIV en 2010 et est autorisée au niveau européen depuis 2011.
Dernièrement, les travaux réalisés sur l'électrodialyse ont porté sur ses potentialités de désacidification des vins (hausse de pH). En effet, certaines régions viticoles cultivent pour des raisons climatiques des cépages spécifiques qui produisent des moûts fortement acides et par conséquent produisent des vins à pH très bas. Ces basses valeurs de pH sont dues aux fortes concentrations en acides organiques. Ainsi, des études ont été réalisées sur des vins blancs et rouges français et complétées par des expérimentations sur un vin québécois. Elles ont mis en évidence à l'échelle laboratoire l'extraction sélective de ces acides grâce à un empilement associant des membranes anioniques et bipolaires, augmentant ainsi le pH. L'intérêt de l'électrodialyse pour la désacidification des vins était ainsi démontré, ajoutant ainsi une nouvelle fonction au procédé. Cette application a été admise en 2012 par l'OIV.
Le procédé d'électrodialyse permet ainsi, avec le même appareil, équipé d'empilements de membranes sous différentes configurations, et de capteurs adaptés (conductimètre, pH-mètre) d'assurer des traitements contrôlés de stabilisation tartrique, et dorénavant d'ajustement de pH. Pilotables, les équipements mis au point permettent d'atteindre très précisément les valeurs cibles pour une qualité parfaitement maîtrisée des vins, et sans aucun ajout d'intrant.

41 – Relationship among instrumental texture properties, phenolic composition and postharvest dehydration kinetics of wine grapes

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The postharvest dehydration process induces qualitative and quantitative changes in the chemical composition of the grapes.[1] Furthermore, thermally processed biomaterials support a texture degradation closely related to enzymatic and non-enzymatic changes in the cell wall pectin.[2] The texture and composition are cultivar dependent, and are strongly related to the dehydration rate that is influenced by the thermohygrometric conditions used.[1,3] Few works have been performed on the incidence of the mechanical properties of winegrapes on the postharvest dehydration process. Therefore, the aims of this work were first to study the effect of the skin hardness of fresh grapes, the winegrape variety and the thermohygrometric conditions on the postharvest dehydration kinetics of the grape, and second to evaluate the possibility of using instrumental texture parameters to manage the off-vine withering process.
The skin hardness of fresh grapes, expressed as break force (F_{sk}), influenced the dehydration kinetics under controlled thermohygrometric conditions (+16°C, 60% RH), particularly for the ripest berries (274±8 and 242±8 g/L reducing sugars for Moscato bianco and Erbaluce, respectively), although this effect was varietal dependent. Two white winegrape varieties, Moscato bianco (F_{sk} = 0.468±0.107N) and Erbaluce (F_{sk} = 0.795±0.182N), and three black varieties, Corvinone (F_{sk} = 0.784±0.124N), Corvina (F_{sk} = 0.827±0.194N) and Freisa (F_{sk} = 0.859±0.163N), showed average daily weight loss percentages (ADWL%) of about 1.7%, 1.0%, 0.7%, 0.8% and 1.2%, respectively. A lower skin break force, a faster weight loss. The following step was to evaluate the influence of different skin hardness values (F_{sk} = 0.372–0.675N and F_{sk} = 0.681–1.233N) for Erbaluce fresh grape berries dehydrated at four different environmental conditions (A = +15°C, 55% RH; B = +18°C, 75% RH; C = +28°C, 40% RH; D= uncontrolled) on the weight loss rate. Differences were found in the dehydration kinetics according to increased temperature (ADWL% = 4.1) or relative humidity (ADWL% = 0.8), which were significant between soft and hard skins only under the lowest dehydration temperature.
The influence of different environmental conditions on the changes in instrumental texture properties and in the phenolic composition of Corvina winegrapes during postharvest withering was then studied. At any controlled condition tested (A = +15°C, 45% RH; B = +8°C, 45% RH; C = +15°C, 80% RH), significant variations were observed, but the extension of the changes depended on the parameter and dehydration kinetics. Particularly, the skin break force and pedicel detachment force decreased significantly (maximum variation of –0.258 and –1.306 N, respectively), whereas total flavonoids of the skin and seeds, and proanthocyanidins and low weight flavanols of the seeds increased (maximum variation of +1483, +733, +1022 and +469 mg/kg, respectively). These results highlighted the necessity of the careful control of the skin break force in modelling the dehydration kinetics, and that the mechanical properties and phenolic composition of the withered grapes can be partly modulated during the dehydration process under controlled thermohygrometric conditions. This fact is of great relevance in the production of dessert wines.

Riassunto in italiano

Relazioni tra proprietà meccaniche, composizione fenolica e cinetica di disidratazione di uve sottoposte ad appassimento
Il processo di appassimento dopo raccolta induce variazioni qualitative e quantitative nella composizione chimica delle uve.[1] In aggiunta, la temperatura del processo supporta una

variazione delle caratteristiche meccaniche per degradazione enzimatica e non enzimatica delle pectine componenti le pareti cellulari.[2] Le proprietà meccaniche e compositive delle uve sono dipendenti dalla varietà, e sono correlate fortemente alla velocità di disidratazione, la quale è influenzata dalle condizioni ambientali (temperatura, umidità) utilizzate.[1,3] Al momento attuale pochi lavori hanno valutato l'influenza delle caratteristiche fisiche delle uve sul processo di appassimento. Gli scopi di questo lavoro sono innanzi tutto di studiare la l'effetto della durezza della buccia (forza di rottura) di uve non appassite, della varietà e delle cinetiche di appassimento in condizioni ambientali controllate, e successivamente di valutare la possibilità di tenere conto delle proprietà meccaniche delle uve per gestire il processo di appassimento.
Il parametro di durezza della buccia, espresso come forza di rottura (F_{sk}), è risultato influenzante la cinetica di disidratazione delle uve sottoposte ad appassimento controllato (16°C, 60% RH), in particolare per le uve testate più mature (274±8 e 242±8 g/L di zuccheri riduttori, rispettivamente per le cultivar Moscato Bianco ed Erbaluce, sebbene sia risultato dipendente dalla varietà utilizzata. Le varietà a bacca bianca Moscato bianco (F_{sk} = 0.468±0.107N) ed Erbaluce (F_{sk} = 0,795±0,182N), e tre varietà a bacca nera, Corvinone (F_{sk} = 0,784±0,124N), Corvina (F_{sk} = 0,827±0,194N) e Freisa (F_{sk} = 0.859±0.163N), hanno mostrato rispettivamente valori di calo peso giornaliero percentuali di circa 1,7, 1,0, 0,7, 0,8 e 1,2%. I campioni con una minore forza di rottura della buccia sono risultati quelli con una velocità di disidratazione maggiore. Il passo successivo ha riguardato la valutazione di due gruppi di acini della cultivar Erbaluce suddivisi in base alla forza di rottura della buccia (F_{sk} = 0,372–0,675N e F_{sk} = 0,681–1,233N), sottoposti a differenti condizioni ambientali di appassimento (A = 15°C, 55% UR; B = 18°C, 75% UR; C = 28°C, 40% UR; D = non controllato) al fine di valutare la cinetica del calo peso durante il processo. Sono state trovate delle differenze nella cinetica sia per quanto riguarda il test a temperatura maggiore (media giornaliera 4,1%) che a umidità maggiore (media giornaliera 0,8%), risultate significative tra i due gruppi separati in base alla forza di rottura della buccia solo nei test a temperatura di appassimento più bassa.
È stata anche studiata l'influenza delle differenti condizioni ambientali di appassimento sulle variazioni nelle proprietà meccaniche di uve Corvina durante il processo. A tutte le condizioni di temperatura e umidità testate (A = 15°C, 45% UR; B = 8°C, 45% UR; C = 15°C, 80% UR), sono state osservate variazioni significative, sebbene l'entità della variazione dipenda dal parametro analizzato. La forza di rottura della buccia e la forza di distacco del pedicello sono diminuite significativamente (variazione minima rispettivamente di -0,258 e -1,306 N), mentre gli indici legati alla composizione fenolica quali flavonoidi totali delle bucce e vinaccioli, proantocianidine e flavanoli a bassa massa molecolare dei vinaccioli sono risultati più elevati (variazione massima rispettivamente di 1483, 733, 1022 e 469 mg/kg). Questi risultati evidenziano la possibilità di valutare la forza di rottura della buccia delle uve da sottoporre ad appassimento al fine di modellizzare le cinetiche di appassimento, e che le proprietà meccaniche e la composizione fenolica delle uve appassite possono essere in parte modulate in processi di appassimento a condizioni ambientali controllate. Questi aspetti sono di particolare importanza nella produzione di vini passiti.

42 – Interspecies gene transfer in wine yeasts: a safe alternative to GMO technology

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From the many yeasts participating in vinification of grape must, *Saccharomyces cerevisiae* and *S. uvarum* play the most important roles. In natural wine yeast populations, chimeric („hybrid") strains also occur whose genomes consist of mosaics from these two species and/or *S. kudriavzevii*. The chimeric genomes most probably arise from rare interspecies hybrids by rearrangement and gradual elimination of large parts of the partner genomes („postzygotic genome stabilisation", reviewed in Sipiczki, FEMS Yeast Lett., 2008). As demonstrated by Italian researchers and our team, wine strains of these species can be hybridised under laboratory conditions by sexual mating of their haploid cells, and the hybrids may have enological properties superior to both parents. However, these interspecies hybrids are sterile and unable to produce viable segregants with chimeric genomes similar to those observed in nature. To break up the sterility barrier, we developed a method (Pfliegler et al., FEMS Yeast Lett., 2012), which is based on hybridization of diploids instead of haploids and on the subsequent elimination of the chromosome that carries the *MAT* locus from one of the partner genomes. The resulting alloaneuploid is fertile and produces stabile chimeric segregants with an (almost) complete genome of one partner and genes from the other partner. This method allows the improvement of wine yeasts by transferring genes between the above species without the application of GMO techniques.

TORNA ALL'ELENCO – BACK TO THE TOP

43 – Study of gene expression: a tool to understand the behaviour of Oenococcus oeni in wine

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The correct performance of wine malolactic fermentation (MLF) depends on the metabolic characteristics of the *Oenococcus oeni* strain/s responsible for this process. The criteria for strain selection include mainly the search for an efficient malolactic activity and a low production of undesirable compounds, such as acetic acid.
The main objective of this work was to evaluate the usefulness of the analysis of gene expression for a better understanding of the *O. oeni* strains performance under wine conditions. The study included the evaluation of different *O. oeni* strains by means of measuring the evolution of metabolites that may have an impact on organoleptic qualities, the related enzymatic activities and their gene expression, and the transcriptional response of several stress responsive genes. Four *O. oeni* strains were selected for this work, showing positive or negative traits in terms of malolactic performance, the citric acid use related to acetic acid production, and stress adaptation. The results revealed a good correlation between a higher initial expression of both the malolactic enzyme and the encoding gene *mleA* and a faster MLF. Among citrate metabolism genes, citrate lyase gene (*citE*) revealed relevant changes indicating citrate consumption during MLF. Related to acetic acid production, the increase of acetate kinase gene (*ackA*) expression was in accordance with acetic acid generation. On the other hand, the increment of acetolactate decarboxylase gene (*alsD*) transcriptional levels may be linked to the low production of diacetyl. These differences in the expression patterns were in agreement with the differences in the content of compounds affecting organoleptic characteristics, such as acetate and diacetyl. Moreover, the strains that performed best in wine-like conditions presented a much higher relative expression of several genes coding for stress proteins, particularly *hsp18*, *clpP*, *ctsR* and *rmlB*.
Finally, the interstrain comparison of the transcriptional levels of selected genes at different times during MLF proved to be a good indicator of the positive or negative strain performance. In conclusion, the analysis of gene expression of representative genes is an useful tool for the characterisation and the selection of malolactic starters

50 – A cellar of bioclimatic architecture experiments geothermy

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A cellar " Celler Batlle " of Gramona, located in Sant Sadurni d' Anoia, in Spain, in the D.O. (Denominación de Origen) of Penedès near Barcelona, is a recent building (2001) which was designed from the beginning according to a logic of bioclimatic architecture: buried in 90 %, using at most the natural light and ventilation. Once the ship was operational, the second phase was focused on the outer arrangements (2006), developing at most the ecological and œnotouristic potential of the site: integration of the building by gardens of native plants settled in the roof, organization of pedagogical circuits, development of the biodiversity in gardens, on banks, and in vineyards (BIODIVINE program). The third phase (2010-2012) focuses on the development of the energy autonomy of the cellar. It was decided to develop an installation based on geothermy.
The project consists in changing the system of conventional production of cold which serves during the process of wine making by a system based on geothermic heat pumps to improve the efficiency. The residual free energy, is used for the heating of offices and laboratories. The first stage consists of an energy audit which allows to diagnose needs: quantity, period, localization …
Geothermy allows to use the energy of the natural heat of the under ground, according to the depth of the perforing, the difference between the outside temperature and the temperature under ground being more or less important. A low-temperature system (< 30, < 200m of depth) is set up.
Two heat pumps of 40W act on two tanks of inertia, one for the production of cold and the other one where scatters the heat resulting from the process of cooling. The geothermic heat exchanger, constituted by 20 wells of 120m each (2400m all in all) allows to ensure the stability of the balance between absorbed energy and lost energy.
The tank of cold water ensures:
- The cold for the fermentation and the preservation of the must in tanks.
- The air conditioning of offices and the bottling room
The tank of warm water allows:
- to warm the offices and the bottling room from the ground by re-using the residual heating of the cooling machines
- to dissipate the calorific energy towards the wells of geothermal science in summer.
The system intervenes on 40 % of the energy expenditure of the cellar, by covering them in 50 %. This brings to a reduction of 20 % of the annual global energy expenditure. The amortization of the investment is considered within 8/9 years approx. The installation ended in July, 2012, on time for the grape harvest. The energy saving was 44 %, with an upper efficiency on 55 % compared to the previous machines of cooling, and the CO2 emissions lowered down to 10.315kg. Considering the working comfort, the wished temperatures were more quickly obtained and were stabilized in the duration. The control of fermentation of the must was sharply improved.
In conclusion, the geothermal science is an interesting energy to be developed in cellars, because it does not create difficulties either phonic, or visual (wells are almost invisible in the landscape), it is a green energy which decreases the carbon footprint and the energy bill of the cellar.

Resumé en français

Une cave de conception bioclimatique expérimente la géothermie
La cave « Celler Batlle » de Gramona, située à Sant Sadurni d'Anoia, en Espagne, dans l'appellation du Penedès près de Barcelone, est un bâtiment récent (2001) qui fut conçu dès le départ selon une logique d'architecture bioclimatique : enterrée à 90%, utilisant au maximum la ventilation et la lumière naturelles. Une fois le bâtiment opérationnel, une deuxième phase a été engagée sur les aménagements extérieurs (2006), développant au maximum le potentiel écologique et œnotouristique du site : intégration du bâtiment

par des jardins de plantes autochtones installés en toiture végétale, mise en place d'un circuit pédagogique, développement de la biodiversité dans les jardins, sur les talus, et dans les vignes (programme BIODIVINE).
La troisième phase (2010-2012) se centre sur le développement de l'autonomie énergétique de la cave. Il fut décidé de s'engager dans une installation basée sur la géothermie.
Le projet consiste à changer le système de production de froid industriel conventionnel qui sert lors du processus de vinification du raisin par un système basé sur des pompes à chaleur géothermique pour en améliorer l'efficacité. L'énergie résiduelle, gratuite, est utilisée pour le chauffage des bureaux et laboratoires.
La première étape consiste en un audit énergétique qui permet de diagnostiquer les besoins : quantité, période, localisation…
La géothermie permet d'utiliser l'énergie de la chaleur naturelle du sous sol, selon la profondeur de la perforation, la différence entre la température extérieure et la température du sous sol étant plus ou moins importante. Il est mis en place un système basse température (<30ºC, <200m de profondeur).
Deux pompes à chaleur de 40W agissent sur deux cuves d'inertie, une pour la production de froid et l'autre où se disperse la chaleur résultant du processus de refroidissement. L'échangeur géothermique, constitué de 20 puits de 120m chacun (2400m au total) permet d'assurer la stabilité de l'équilibre entre énergie absorbée et énergie dissipée.
La cuve d'eau froide permet d'assurer :
- le froid industriel pour la fermentation et la conservation du moût dans les cuves.
- le froid industriel circulant dans le serpentin qui permet le refroidissement du raisin à son entrée dans la cave.
- la climatisation des bureaux et de la salle d'embouteillage
La cuve d'eau chaude permet de :
- chauffer les bureaux et la salle d'embouteillage par le sol en réutilisant le chauffage résiduel des machines de refroidissement
- dissiper en été l'énergie calorifique vers les puits de géothermie.
Le système intervient sur 40% des dépenses énergétiques de la cave, en les couvrant à 50%. Ce qui revient à une baisse de 20% des dépenses énergétiques globales annuelles. L'amortissement de l'investissement est estimé à environ 8/9 ans. L'installation s'est achevée en juillet 2012, à temps pour la vendange. L'économie d'énergie a été de 44%, avec un rendement supérieur de 55% comparé aux machines de refroidissement antérieures, et les émissions de CO2 on baissé de 10.315kg. Au niveau du confort de travail, les températures désirées furent atteintes plus rapidement et ont été stabilisées sur la durée. Le contrôle de fermentation du moût a été nettement amélioré.
En conclusion, la géothermie est une énergie intéressante à développer dans les caves, car elle ne crée pas gêne ni phonique, ni visuelle (les puits sont quasiment invisibles dans le paysage), c'est une énergie verte qui permet de diminuer l'empreinte carbone et de diminuer la facture énergétique de la cave.

53 – Yeast COQ1 gene acts as a geraniol /nerolidol synthase in de novo synthesis of terpene compounds in wine production by Saccharomyces cerevisiae

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Terpenes, typical components of flowers and fruits, are also present as free and glycosylated conjugates in many wine grape varieties of *Vitis vinifera*. Hence, when these compounds are present in wine they are considered to be originated from grapes and not as fermentation derivatives.
However, the biosynthesis of mono and sesquiterpenes by *Saccharomyces cerevisiae* in the absence of grape derived precursors has been recently reported to be of *de novo* origin in wine yeast strains.[1] COQ1 was proposed as encoding a putative geranyl pyrophosphate synthase in the yeast mitochondria.
In this work it was proved that COQ1 overexpression in wild type and mutant strains of *S. cerevisiae* enhance production of geraniol and nerolidol by two and four times respectively. At exponential growth conditions, COQ1 gene participates in the isoprenoids biosynthesis fulfilling not only the geranyl pyrophosphate synthase function but also a nerolidol synthase activity.
Intraspecific variability found, both in native *S. cerevisiae* (new SNPs detected) and metabolic engineering of COQ1 gene, could impact on the production of wine aroma, flavors and fragrances, human health and plant protection.

Resúmen en castillano

El gen COQ1 de levaduras actúa como una geraniol /nerolidol sintasa en la síntesis *de novo* synthesis de terpenos durante la producción de vinos por *Saccharomyces cerevisiae*
Los terpenos, componentes característicos de las flores y frutas, se encuentran también como componentes libres y glicoconjugados en uvas de algunas variedades de *Vitis vinifera*. En consecuencia, cuando estos compuestos están presentes en el vino se considera que se originan a partir de uvas y no como derivados de la fermentación.
Sin embargo, recientemente se ha reportado la biosíntesis de mono y sesquiterpenos por *Saccharomyces cerevisiae*, en ausencia de precursores provenientes de la uva, como un proceso de novo.[1] Se ha propuesto que COQ1 codifica una geranilpirofosfato sintasa en la moitocondria de las levaduras.
En este trabajo se demostró que la sobre-expresión de COQ1 en cepas salvajes y mutantes de *S. cerevisiae* incrementa el contenido de geraniol y nerolidol 2 y 4 veces respectivamente.
En condiciones de crecimiento exponencial el gen COQ1 participa en la biosíntesis de isoprenoides cumpliendo no sólo una función de geranilpirofosfato sintasa sino también una actividad nerolidol sintasa.
La variabilidad intraespecífica encontrada tanto en cepas nativas de *S. cerevisiae* (nuevos SNPs) y la ingeniería metabólica del gen COQ1 podría impactar en la producción de aromas de vinos, aromas y fragancias, en la salud humana y en protección vegetal.

54 – Use of ATR-FTIR spectroscopy to monitor alcoholic fermentations and control the physiological state of yeast

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Infrared spectroscopy (FT-NIR) is a powerful technique for the differentiation and identification of vegetative cells and spores. Conventional culture techniques are time consuming and may underestimate the number of viable bacteria. There are interesting alternatives to the traditional methods such as flow cytometry but they are expensive and difficult to use. Developing a simple, rapid, reproducible and sensitive infrared spectroscopy method to study yeast during grape must fermentation, will be a breakthrough for wine industry, who is in need for applying a rapid method to detect microorganisms in order to prevent stop fermentations and cross contaminations.
Infrared spectroscopy techniques combined with multivariate analysis could meet these demands.
FT-NIR has been recently used for wine industry as a fast technique for routine control parameters such as pH, total acidity, volatile acidity and alcoholic degree. Detection and identification of microorganisms in food products is a new application and we have developed a protocol to analyze directly the yeast pellet. Microorganisms involved in winemaking process are mainly yeasts (alcoholic fermentation), lactic bacteria (malolactic fermentation) and acid acetic bacteria (wine oxidation and degradation).
The main objective of this research is to evaluate the potential of using FT-NIR to discriminate and classify yeast strains and analyze their chemical changes and their physiological state during a fermentation process. The use of powerful supervised pattern recognition techniques such as soft independent modeling class analogy (SIMCA) has allowed us to differentiate these changes within *Saccharomyces cerevisiae* strains. FT-NIR provides a simple, rapid and accurate technique for studying yeasts during the fermentation process and can be used to detect secondary fermentations produced by undesirable microorganisms. The information provided in this research will attract much attention from the scientific and industrial communities.
Four commercial strains of *S. cerevisiae* were grown in Chardonnay and Garnatxa Blanca must for up to 198 h, 150ml of must was inoculated and fermentated at 17ºC in termostatized water bath. In each case, the inoculum was prepared according to the instructions provided by the supplier to reach an initial concentration of $1 \cdot 10^8$ cfu/mL S. cerevisiae in must. Fermented samples were centrifuged at 10.000 rpm for 10 minutes and washed twice 0.85% with saline solution. Pellets were finally deposited directly to the ATR ZiSn crystal. Spectra were collected using a Nicolet iS10 FT-NIR spectrometer (Thermo Fisher), from 4000 to 800 cm^{-1} with a resolution of 4 cm^{-1} coadding 128 scans to improve the signal-to-noise ratio**.** Data obtained were analyzed by a multivariate analysis technique, soft independent modeling of class analogy (SIMCA). *S. cerevisiae* strains were discriminated mainly due to the difference in their cell wall composition and classified depending on their physiological state (exponential and stationary phase).

Resúmen en castillano

Uso de la espectroscopia de infrarojo ATR-FTIR para la monitorización de fermentaciónes alcohólicas y control del estado fisiológico de las levaduras

La espectroscopía de infrarrojo (FT-NIR) es una técnica poderosa para la diferenciación e identificación de células vegetativas y esporas. Las técnicas convencionales de cultivo son laboriosas y consumidoras de tiempo y pueden subestimar el número de bacterias viables. Hay alternativas interesantes a los métodos tradicionales tales como la citometría de flujo, pero son caras y difíciles de usar. El desarrollo de un método simple, rápido, reproducible y sensible como la espectroscopia de infrarrojo para estudiar la levadura durante la fermentación del mosto de uva supondría un gran avance para la industria del vino, que tiene la necesidad de disponer de un método rápido para detectar microorganismos con el fin de evitar paradas de

fermentaciones y contaminaciones cruzadas. La técnica de la espectroscopia de infrarrojo combinada con un análisis multivariante podría satisfacer estas demandas del sector.
La técnica de FT-NIR se ha utilizado recientemente para la industria del vino como una técnica rápida para el análisis de los parámetros de control de rutinarios, tales como el pH, acidez total, acidez volátil y grado alcohólico. La detección e identificación de microorganismos en los productos alimenticios es una aplicación nueva, y se ha desarrollado un protocolo para analizar directamente el pellet. Los microorganismos que intervienen en el proceso de elaboración del vino son principalmente las levaduras (fermentación alcohólica), las bacterias lácticas (fermentación maloláctica) y ácido acético (bacterias oxidación del vino y la degradación).
El objetivo principal de esta investigación es evaluar el potencial de la técnica de FT-NIR para distinguir y clasificar las cepas de levadura así como analizar sus cambios químicos y su estado fisiológico durante el proceso de fermentación de forma rápida. El uso de poderosas técnicas de análisis de datos como *soft independent modeling class analogy* (SIMCA) nos ha permitido diferenciar estos cambios dentro de cepas *Saccharomyces cerevisiae*.
La espectroscopía de FT-NIR proporciona una técnica simple, rápida y precisa para estudiar las levaduras durante el proceso de fermentación y se puede utilizar para detectar fermentaciones secundarias producidas por microorganismos no deseados. La información proporcionada en esta investigación atraerá mucha atención de la comunidad científica e industrial del sector del vino.
Cuatro cepas comerciales de *S. cerevisiae* se cultivaron en mostos de Chardonnay y Garnacha Blanca hasta 198 h, 150 ml de mosto se inocularon y se fermentaron a 17 º C en un baño de agua termostatizada. En cada caso, el inoculo se preparó de acuerdo con las instrucciones proporcionadas por el proveedor para alcanzar una concentración inicial de $1 \cdot 10^8$ ufc / ml de *S. cerevisiae* en el mosto. Las muestras fermentadas se centrifugaron a 10000 rpm durante 10 minutos, lavandolas dos veces con una solución salina al 0,85%. Los pellets fueron depositados directamente en el cristal del ATR de ZiSn para la adquisición de los espectros. Los espectros se recogieron usando el espectrómetro FT-NIR Nicolet iS10 (Thermo Fisher), des de 4000 a 800 cm^{-1} con una resolución de 4 cm^{-1} y superposición de 128 exploraciones para mejorar la relación señal-ruido. Los datos obtenidos se analizaron mediante técnicas de análisis multivariante, soft independent modeling class analogy (SIMCA). Las cepas de *S. cerevisiae* se discriminaron debido principalmente a la diferencia en la composición de su pared celular y se clasificaron en función de su estado fisiológico (fase exponencial y estacionaria).

55 – E-CO_2: recovery and re-use of CO_2 from wine fermentations

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Fermentation is a natural process that enables the transformation of must in wine, through the conversion of sugars in ethyl-alcohol and carbon-dioxide (CO_2).
Stoichiometrically, with 45 million hectoliters of wine with an average alcohol content of 12% v/v, annually produced in Italy, it is possible to estimate the huge production of 45,000 tons.
Every year the production of wine leads to the release of a large amount of carbon dioxide in the atmosphere and this contributes to the amount of greenhouse gases.
The CO_2 reabsorbed by photosynthesis is only a small part of the total amount produced; therefore it is necessary to reduce this issue.
In order to succeed in this challenge, a project called E-CO_2 was kicked off in June 2011: it aims at the considerable reduction of emissions in the step of fermentation. The CO_2 is collected, purified and compressed, restoring the important value of the *resource* (which is free because it results from process waste and left into the atmosphere) and the project assesses the CO_2 re-use in various ways in the wine industry as well as in other sectors that usually consume large quantities.
The carbon dioxide has an extremely important market, in continuous expansion, and it is used for operations such as carbonation of beverages, creation of modified/protected atmosphere for foods or in wine industry operations.
A further possible application in agriculture and the nutraceutical field is its use for feeding photosynthetic organisms such as unicellular algae.
In winemaking, CO_2 is very useful for the control of oxidation and extraction of aromatic compounds.
Wineries are direct users of technical gases and they may create an important synergy with gas producers, creating a 'a CO_2 bank' for self-use throughout the year. This could reduce the CO_2 production from combustion and chemical reactions, which are the present ways of production.
However the generous resource as the one from winery fermentations has never been seriously evaluated so far due to the periodicity; a so large-scale production in a short time range would require the construction of huge facilities with heavy economic impact, with risk of depreciation.
In E-CO_2 project, the application of technologies for collection and storage technologies, already widespread and entreched in other sectors (as breweries), was evaluated and it has been observed that the wine sector represents a real innovation.
Chemical analyses which were carried out showed that the purity of the CO_2 from alcoholic fermentation is extremely close to that required for international marketing as a gas with a “food grade” quality and a mild purification would be sufficient.
Additionally, among the compounds that “contaminate” the wine CO_2 , it is possible to find also fermentation aromas, which, if properly condensed, may serve as an additional resource to be evaluated as a food ingredient.
The project has received the strong attention of several actors in Verona’s wine scene as well as of retailers of technical gases.

Riassunto in italiano

E-CO_2: recupero e riutilizzo della CO_2 di fermentazione
La fermentazione è un processo naturale che consente di trasformare il mosto in vino, attraverso la conversione degli zuccheri presenti in alcol etilico e anidride carbonica (CO_2).
Dal punto di vista stechiometrico, da 45 milioni di ettolitri di vino con una gradazione media di 12% v/v, prodotti annualmente in Italia, è possibile stimare una emissione di CO_2 pari a 450.000 ton. Ogni anno il processo di vinificazione porta, quindi, alla liberazione di ingenti quantità di CO_2 nell’atmosfera intensificando l’effetto serra.

TORNA ALL’ELENCO – BACK TO THE TOP

Anche se parte della CO_2 viene riassorbita nei vigneti attraverso la fotosintesi, questo processo non è sufficiente a bilanciare tutta la CO_2 prodotta in fase di fermentazione: è pertanto necessario individuare soluzioni efficaci per ridurne l'emissione.
Al fine di affrontare questa sfida, nel giugno 2011 è stato avviato un progetto denominato E-CO_2 che ha proprio l'obiettivo di ridurre la liberazione in atmosfera di CO_2: il gas viene captato in uscita dalle vasche durante la fermentazione, purificato e compresso trasformandolo in *risorsa*, per un possibile riutilizzo nel settore enologico, ma anche in altri settori dove viene già impiegato per svariati scopi.
Il mercato della CO_2 è estremamente importante nel settore della chimica e tecnologia industriale ed è in continua espansione in campo alimentare per operazioni come la gasatura di bevande e la creazione di atmosfere modificate/protette per la conservazione di alimenti. Ulteriori possibili applicazioni in campo agrario e nutraceutico riguardano la nutrizione di organismi fotosintetici come le alghe unicellulari. In ambito enologico la CO_2 è utile per il controllo delle ossidazioni e dell'estrazione delle sostanze aromatiche. Le cantine stesse sono, quindi, dirette utilizzatrici di gas tecnici e potrebbero trovare una importante sinergia con i produttori di gas, creando una 'banca della CO_2' per utilizzarla in modo continuativo nel corso dell'anno. Si potrebbero così ridurre le produzioni di CO_2 da combustioni e reazioni chimiche.
È tuttavia evidente che una risorsa generosa come quella delle fermentazioni vinarie non è mai stata pienamente presa in considerazione per motivi legati alla stagionalità; infatti grandi produzioni in un ristretto periodo di tempo comportano la realizzazione di grossi impianti di raccolta con pesanti incidenze economiche a livello di ammortamenti.
Nel progetto è stata quindi valutata in modo approfondito l'applicazione di tecnologie di raccolta e immagazzinamento già diffuse e consolidate in altri settori non stagionali (birrifici), ma che nel comparto vitivinicolo rappresentano una reale innovazione.
Le analisi chimiche effettuate hanno evidenziato che la purezza della CO_2 da fermentazione alcolica vinaria è estremamente vicina a quella richiesta dai disciplinari internazionali per la commercializzazione come gas *food grade* e che la qualità richiesta può essere soddisfatta con una blanda purificazione.
Tra i composti che contaminano la CO_2 enologica sono presenti anche aromi di fermentazione, che, se opportunamente condensati, potrebbero rappresentare un ulteriore risorsa da valorizzare come ingrediente alimentare.
Il progetto ha raccolto la forte attenzione di diverse realtà locali del panorama vitivinicolo veronese oltre che rivenditori di gas tecnici nazionali e internazionali.

57 – New dangers in enology: Staphylococcus epidermidis producing histamine in wines

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Biogenic amines are undesirable compounds in wine synthesized by lactic acid bacteria. Aside from being toxic to humans, biogenic amines depreciate the quality of wines.
This study began with the arrival to the laboratory of a sample of wine from *Tempranillo*, whose histamine concentration was above 10 mg/L. The aim was the isolation, identification and characterization of those microorganisms responsible for the synthesis of histamine. Four strains of *Oenococcus oeni* and two strains of microorganisms whose 16S gene sequence identified as *Staphylococcus epidermidis* were isolated. To discard the possibility of environmental contamination the isolation was repeated, obtaining again such strains of *Staphylococcus epidermidis* from the wine.
This microorganism is described as histamine producer in various foods, but never before it was isolated from wine neither its ability to produce histamine in wine had been reported. Then we proceeded to carry out the relevant tests to determine which of the different isolates was responsible for histamine production in this wine. Such assays showed as only one histamine producer strain of *Staphylococcus epidermidis*, not showing any of the isolates from *Oenococcus oeni* such ability. Surprisingly, this *Staphylococcus epidermidis* strain was not only able to produce histamine, but also putrescine and cadaverine. This shows the need for the asepsis in the cellars and care in manufacturing processes, warning of the existence of this type of organisms in wine, although sporadic, could have a major negative impact on the final quality of the wine.

Resúmen en castillano

Nuevos peligros en enología: Staphylococcus epidermidis productor de histamina en vinos
Las aminas biógenas son compuestos indeseables en el vino sintetizados por las bacterias lácticas. Además de ser tóxicas para el ser humano, deprecian la calidad de los vinos.
Este estudio comenzó con la llegada al laboratorio de una muestra de vino de la variedad Tempranillo, cuya concentración de histamina era superior a 10 mg/L. La finalidad fue el aislamiento, identificación y tipificación del microorganismo responsable de dicha síntesis de histamina. A partir de ese vino se obtuvieron cuatro cepas de *Oenococcus oeni* y dos cepas de microorganismos cuya secuenciación del gen 16S identificó como *Staphylococcu epidermidis*. Ante la posibilidad de una contaminación ambiental se repitió el proceso de aislamiento, constatando repetidamente la existencia de dichas cepas de *Staphylococcus epidermidis* en el vino de partida.
Este microorganismo está descrito como productor de histamina en diferentes alimentos, no existiendo ninguna referencia bibliográfica del aislamiento de dicho microorganismo en vino ni de su capacidad de producir histamina en el mismo. Posteriormente se procedió a la realización de los ensayos pertinentes para determinar cuál de los diferentes aislados era el responsable de la producción de histamina en vino. Dicho ensayos mostraron como único productor a una de las cepas de *Staphylococcus epidermidis*, no mostrando ninguno de los aislados de *Oenococcus oeni*

tal capacidad. Cabe destacar de dicha cepa de *Staphylococcus epidermidis* no sólo fue capaz de producir histamina, sino putrescina y cadaverina.
Este trabajo demuestra la necesidad de la asepsia en las bodegas y el cuidado en los procesos de elaboración, advirtiendo de la existencia de microorganismos no descritos antes en el vino que, aunque esporádicos, podrían tener un gran impacto negativo en la calidad final del vino donde se encuentre.

58 – Influence of oxidation mechanisms during must elaboration on the aromatic quality of Melon B. and Sauvignon Blanc wines

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Must elaboration is considered as a key step in white or rosé winemaking processes. During this step, enzymatic oxidations occur that are considered as detrimental for future wine's quality. For varietal thiols which are key aromas of several wines (1) technical observations mention that must polyphenols oxidation leads to their decrease in wines. However, at this step, they are under precursor's forms, i.e. conjugated to amino-acids, and then theorically not sensitive to oxidation. Thus the mechanisms that could explain this loss are not so clear.
In order to study the evolution of thiols precursors during must oxidation, 2 grapes varieties -Melon B. and Sauvignon Blanc - were chosen, as they are typical of the Loire Valley and representative of two behaviors in regard with oxidation (2). The Stable Isotope Dilution Assay of the cysteinylated and glutathionylated conjugates of 3-mercaptohexanol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP) was developed, that required the synthesis of labeled molecules. They were used as analytical standards but also as tracers for relationship studies and allowed us to formally identified the *S*-3-(hexan-1-ol)-glutathione (G3MH) which was up to now only tentatively identified (3). Those labeled compounds were also used to establish the precursor's role of the G3MH and the *S*-4-(4-methylpentan-2-one)-glutathione (G4MMP) during the alcoholic fermentation. Their conversion yields into thiols were determined close to 4.4 and 0.3 % respectively.
Lab studies showed that the cysteinylated conjugates were not affected by oxidative reactions occurring in must, as expected in regard to their chemical structures. On the contrary, we observed that G3MH was overproduced, as soon as the reaction of glutathione on the caftaric acid quinones was slowing. Similar observations were performed at industrial scale: the elaboration of Melon B. musts under inert gas appeared not in favor of a G3MH overproduction, and the 3MH levels decreased in wines even if no sensorial impact was pointed out. For Sauvignon Blanc, the thiol potential was not significantly affected, but the 3MH levels nevertheless decreased in the wines produced from the inerted juices. The *E*-(2)-hexenal pathway (4), which importance is obviously minor in the inerted juices, might explain this observation. Thus, under ours conditions, a moderate oxidation of Melon B. and in a certain extend of Sauvignon Blanc must is favorable to the aromatic quality of wines from Loire Valley. Further studies are needed to quantify this moderate oxidation concept, and make it generic.

Resumé en français

L'élaboration des moûts est considérée comme une étape clef de la vinification en blanc et rosé. Durant cette étape, des réactions enzymatiques ont lieu, qui sont souvent jugées comme défavorables à la qualité du futur vin. Pour les thiols variétaux, arômes clefs de nombreux vins, les technologues mentionnent que l'oxydation des polyphénols entraîne leur diminution dans les vins. Pourtant à cette étape, les thiols sont sous forme de précurseurs, c'est-à-dire conjugués à des acides aminées ou peptides, et ne sont ainsi théoriquement pas sensible à l'oxydation. Ainsi les mécanismes qui expliquent cette perte aromatique n'apparaissent pas clairs.
Afin d'étudier l'évolution des précurseurs de thiols durant l'oxydation des moûts, deux cépages, le Melon B. et le Sauvignon Blanc, ont été choisis puisqu'ils sont typique de la vallée de la Loire et représentatif de deux comportement distinct par rapport à l'oxydation. Une méthode de dosage par dilution isotopique des conjugués à la cystéine et au glutathion du 3-mercaptohexanol (3MH) et de la 4-mercapto-4-méthylpentan-2-one (4MMP) a été développée, qui a requis la mise au point de la synthèse de leur analogues deutérés. Ces derniers ont été utilisés à la fois comme standards analytiques mais aussi comme traceurs pour les études de filiation que nous avons menées, et ont permis d'identifier formellement le *S*-3-(hexan-1-ol)-glutathion (G3MH) qui n'avait été identifié jusque là que de manière tentative, ainsi que le rôle de précurseur du G3MH et du *S*-4-(4-methylpentan-2-one)-glutathion (G4MMP) durant la fermentation alcoolique. Les rendements de

conversion de ces conjugués en thiols volatils sont proches de 4,4% et de 0,3 % pour respectivement le G3MH et la G4MMP

Des expérimentations à l'échelle laboratoire ont montré que les conjugués à la cystéine n'étaient pas affectés par les réactions d'oxydation dans les moûts, comme attendu eu égard à leur structure chimique. En revanche, nous avons observé que du G3MH se formait, dès que la réaction entre le glutathion et les quinones de l'acide caftarique ralentissait. Des observations similaires ont été faites à l'échelle industrielle : l'élaboration de moûts de Melon B. sous gaz inerte n'est pas apparue favorable à la formation supplémentaire de G3MH, et les teneurs en 3MH des vins correspondants étaient moindres, même si aucune différence sensorielle n'a pu être mise en évidence. Pour le Sauvignon Blanc, le potentiel en thiols n'a pas été significativement modifié, mais les concentrations en 3MH se sont tout de même avérées plus faibles dans les vins produits à partir des jus inertés. La voie du *E*-(2)-hexenal, de moindre ampleur sous gaz inerte, pourrait expliquer cette observation. Ainsi, dans nos conditions, une oxydation ménagée des moûts de Melon B., et dans une certaine mesure, de ceux de Sauvignon Blanc est favorable à la qualité aromatique de ces vins. Des études ultérieures sont cependant nécessaires pour mieux appréhender et quantifier le concept d'oxydation ménagée, et le rendre ainsi plus générique.