CHEMISTRY AND ANALYSIS OF KEY VOLATILE COMPOUNDS OF WINE AND THEIR PRECURSORS IN GRAPE•

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1. INTRODUCTION

In the headspace of a wineglass, among one to several hundred compounds are present. The concentration of these compounds, as their olfactory detection threshold values, can vary between several hundred milligrams per liter to trace concentrations in the picogram per liter range. Among such compounds, a relatively small number of them, often present at trace concentrations, are considered as key volatile compounds. These compounds do not explain alone all the nuances of wine aroma but they can contribute to some specific notes. Knowledge of these essential pieces in the puzzle of wine olfactory images is so very important especially to understand the evolution of wine aroma and the chemical and biochemical mechanisms in which they are involved.

2. WHAT DO WE LEARN FROM THE PHYSIOLOGY OF OLFACTION?

The consideration of the physiology of flavor perception can be a way for a better definition of these compounds. Briefly, the perception of the wine flavor is first related to a combinatorial response of our olfactory sensory system in relation with the detection of volatile molecules, soluble in the mucus of olfactory epithelium, which are considered as stimuli. Then, there is integration of all sensory informations at the level of consciousness where there is feeling, recognition and description of the flavor (Shepherd. 2006). This mechanism proceeds through different steps (unconscious at the epithelium level, in the olfactory bulb, then conscious in the limbic system, piriform cortex, right orbitofrontal cortex….). At the level of the olfactory bulb, the combinatorial response concerns the activation of glomeruli patterns, depending on the volatile or combination of volatiles detected in the olfactory epithelium by the sensory neurons (LLedo *et al*., 2004; Shepherd, 2006). These patterns are transmitted to the cortex to produce sensations in the field of consciousness. That is, key volatile compounds can be defined as those capable to contribute, through activation patterns, alone or in mixture, as in the case of perceptual interactions, at the brain level to the recognition of specific wine's aroma by wine tasters.

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3. HOW CAN WE PROGRESS IN THE CHARACTERIZATION OF SUCH COMPOUNDS?

Always the methodology considers the selection of representative wines by sensory analysis, then the characterization of the key volatile compounds through molecular identification and quantification in wine. The determination of sensory properties is studied in the presence of the compound alone in various matrices or in a combination with various odorants through reconstitution experiments.

Traditional extraction protocols include liquid-liquid extraction with solvents, liquid-gas extraction as purge and trap technique and vacuum distillation, gas or liquid-solid extraction as SPME (Solid Phase Micro Extraction) and SBSE (Stir Bar Sorptive Extraction). Recently analytical accessories were developed which can accelerate and simplify the extraction protocols, as SPDE (Solid Phase Dynamic Extraction) where the headspace is aspirated with a syringe and compounds interact with a polymer. Then the syringe is placed in the injector of the gas chromatograph (GC) and the compounds are thermally desorbed. Another system is MEPS (Micro Extraction with Packed Sorbent) using a syringe with a polymer (silica grafted C8, 18 or other) allowing the adsorption of the compounds present in the liquid. Then, the polymer is washed with a solvent and the compounds are eluted with a more apolar solvent before injection in the GC. But concerning the identification and quantification of key volatile compounds, as they are usually present at trace concentrations in wines, it is necessary to develop multistep extraction protocols. They include selective extraction or clean up procedures, HPLC fractionations of the extracts (Pineau *et al*., 2009) or derivatization protocols particularly for carbonyl compounds (Culleré *et al*., 2006).

The necessary step for the identification of key volatile compounds is the use, at beginning, of GC coupled to an olfactometric detection. This technique has largely demonstrated over the last twenty years its relevance for evidencing strong odoriferous compounds, reminiscent of the nuances of the wine aroma. But, when the identification of these compounds is considered by coupling gas chromatography with mass spectrometry, the co-elution phenomena do not make possible the direct identification of the compounds implicated. So, monodimensional gas chromatography should be supplemented by multidimensional gas chromatography techniques allowing the transfer of the eluted compounds from the first column on a second capillary of different polarity. Heartcutting method (2DGC) is an elegant approach for increased resolution and sensitivity in selected zones of the chromatogram for the identification or assay of volatile compounds in a complex matrix as wine (Pons *et al*., 2008a). Moreover, comprehensive GC or GCxGC techniques offer powerful resolution capacity with the aim of a global analysis of volatiles (Marriott *et al*., 2009).

Mass spectrometry techniques as chemical ionization and MS/MS detection system can also help to improve the sensitivity and selectivity of the quantification. Moreover, time of flight (TOF) mass spectrometers which possess increased acquisition rate permit to enhance the ability to detect coeluted compounds thanks to a higher chromatographic resolution. Particularly, high resolution mass spectrometry (HRMS) provides precision in mass determination which lowers the ambiguities for the identification of volatile compounds. For example, Sarrazin *et al*. (2010) obtained in dessert wine extracts the high resolution

mass spectra of a trace volatile compound presenting a strong grape fruit odor. This molecular ion at 132,0609 Da permitted to propose the elemental formula $C_6H_{12}OS$ based on a calculated mass of 132, 0605 Da which was coherent with the identification of 3-propyl-1,2-oxathiolane.

4. CHEMISTRY OF SOME IMPACT COMPOUNDS IN WINE

4.1. Some aspects of volatile sulfanyl compounds formation and reactivity

Various examples can be considered to illustrate the specificity of chemical and biochemical reactions in wines contributing to the formation of key volatile compounds or their degradation. Particularly, some thiol (sulfanyl) compounds with powerful aromas are involved in the sensory characteristics of wines from different grape cultivars ('Sauvignon blanc', 'Manseng', 'Cabernet sauvignon'…) as they can exhibit attractive tropical fruit, boxtree, grape fruit aroma at trace concentrations. Other thiols issued from fermentation and contributing to fermentative and ageing wine aroma can be related to cooked meat or torrified coffee flavors. 3-sulfanylhexan-1-ol (3SH) with a powerful odorant reminiscent of citrus is one of the most studied and analyzed compound. The mechanisms involved in its formation, reactivity and stability, constitute a very challenging subject. Particularly, different proportions of 3-sulfanylhexan-1-ol enantiomers, which present different flavour nuances, can be found depending on the type of wine (dry Sauvignon wine or dessert wine obtained after grape botrytization). This compound exists also as aroma precursor in grape, as a conjugate of cystein, which can be metabolized during alcoholic fermentation by *Saccharomyces cerevisiae* to release the volatile thiol. Recently, various research groups (Capone *et al.*, 2010; Kobayashi *et al*., 2010; Roland *et al*., 2010) have assayed a glutathionyl conjugate of 3-sulfanyl hexanol , which was first identified by Peyrot des Gaschons *et al*. in 2002.

Moreover, analysis of S-cystein conjugates of 3-sulfanylhexan-1-ol (P-3SH) by GC as perfluorated acylated derivatives, after their purification from grape must, made it possible to demonstrate an increase of P-3SH in relation with the development of noble rot on grapes due to *Botrytis cinerea* (Thibon *et al.*, 2009).

Sulfanyl compounds are also very reactive in the presence of oxygen and metals (copper, iron) even at trace concentrations. Particularly, 3-sulfanylhexanol and 4-sulfanyl-4-methylpentan-2-one concentrations in a two year old Sauvignon blanc wine were shown to be affected by the closure type (cork closure, synthetic closure, screwcap) in relation to its permeability to oxygen (Lopes *et al.*, 2009). The decrease of thiol concentration in wines after bottling is not directly related to their reactivity with oxygen but more to their reactivity with phenolic compounds in the presence of oxygen (Nikolantonaki *et al*., 2010). Thus, a study in model medium with a composition close to wine, in the presence of oxygen and (+)-catechin or (-)-epicatechin has permitted to demonstrate a diminution of thiol concentration following different kinetics depending on the type of sulfanyl compound, a primary sulfanyl (2-methanefuranthiol) being more reactive than a secondary (3-sulfanylhexan-1-ol) or a tertiary (4-sulfanyl-4-methylpentan-2-one) (fig. 1).

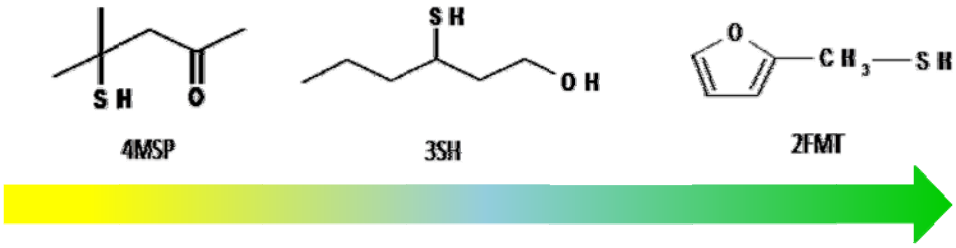

Fig. 1 - Level of reactivity of volatile thiols with flavan-3-ol [(+)-catechin].

4.2. Identification of the disulfide of 3-sulfanylhexanol in dessert wines

The chemical origin of 3-propyl-1,2-oxathiolane identified in dessert wines was investigated. This compound was detected in botrytized dessert wines but not in the dry white wines from the same varieties. Its occurrence in Sauternes wine extracts was shown to result in fact from the thermal oxidative degradation of 3SH disulfide (3,3'-disulfanediyldihexan-1-ol) in the GC injector. To validate this observation, a purification procedure was developed for isolating 3SH disulfide from Sauternes wine. The purified fraction, submitted to derivatization using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, permitted to obtain a signal corresponding to the mass spectrometry pattern of silylated 3SH disulfide. Identification was then confirmed by HRMS. The particular presence of 3SH disulfide in dessert wines is associated to a different chemical reactivity of volatile thiols in these wines, probably due to their modified phenolic composition induced by *B. cinerea*.

4.3. Formation of key volatile compounds related to wine oxidative evolution

The premature evolution of the aroma of some wines is a concern considering the frequent evolution towards oxidative nuances. In the dry white wines, this evolution is related to aromatic notes of wax, honey, nuts. These irreversible phenomena also lead to the evolution of yellow hue (DO_{420}).

Several markers of premature aging of aromatic dry white wines have been identified in the recent years, including sotolon, principally its *S*-enantiomer, a furanone smelling of nuts, curry, and celery (Pons *et al.*, 2008b), phenylacetaldehyde with rose and honey notes, methional presenting boiled potato descriptors (Silva Ferreira *et al.*, 2003; Sarrazin *et al.*, 2007). Regarding sotolon, different pathways for its formation have been proposed in food and wine but there is ambiguity concerning the intermediates in the formation of sotolon in dry white wine relatively to its oxydation. Research in model medium of precursors of sotolon, issued from oxidative degradation of ascorbic acid, permitted to identify 2-ketobutyric acid. In wines, 2-ketobutyric acid is also a product of yeast *S.cerevisiae* metabolism (concentrations in the range 1-15 mg/L). A pathway leading to the chemical formation of sotolon in dry white wines was demonstrated to include an aldol reaction between 2-ketobutyric with acetaldehyde, particularly in the presence of low concentrations of free sulfur dioxide (Pons *et al.*, 2010) (fig. 2). Certain modalities of wine technology, such as wine aging on lees with stirring, help to limit the formation of this compound during aging of bottled wine (Lavigne *et al.*, 2008).

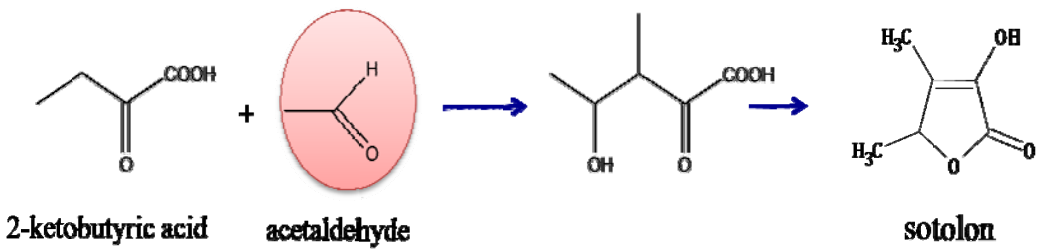

Fig. 2 - Identification of a chemical sotolon pathway, through aldol condensation reaction in dry white wines.

Abstract

A relatively small number of the many volatile substances of wine, often present at trace concentrations, are considered as key volatile compounds. These compounds often exist in grapes under poorly odoriferous or non volatile forms as aroma precursors. Therefore, innovative analytical techniques are essential to identify and quantify them in grape and wine. These analytical techniques are also dedicated to the study of chemical reactions in which these compounds are involved during grape maturation, vinification and ageing. Trough the examples of various wine's aroma compounds, as sulfanyl compounds and their precursors, and compounds related to oxidative ageing aroma, this paper reviews the value of innovative techniques dedicated to the characterization of secondary metabolites. The diversity of the mechanisms implicated in the reactivity and the genesis of such compounds is briefly illustrated.

Literature cited

Capone D.L., Sefton M.A., Yoji H., Jeffery D.W. - 2010 - Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS: resolution and quantitation of diastereomers of 3-S-cysteinylhexan- 1-ol and 3-S-glutathionylhexan-1-ol. *J. Agric. Food Chem*., 58, 1390-1395.

Culleré L., Cacho J., Ferreira V. - 2006 - Validation of an analytical method for the solid phase extraction, in cartridge derivatization and subsequent gas chromatographic-ion trap tandem mass spectrometric determination of 1-octen-3-one in wines at ng.L^{-1} level. *Anal. Chim. Acta*, *53,* 51-57.

Dubourdieu D., Tominaga T. - 2009 - Polyfunctional thiols compounds. In: M.V. Moreno-Arribas, M.C. Polo (eds) *Wine chemistry and biochemistry*. Springer, NY, USA, 275-294.

Kobayashi H., Takase H., Kaneko K., Tanzawa F., Takata R., Suzuki S., Konno T. - 2010 - Analysis of *S*-3-(hexan-1-ol)-glutathione and *S*-3-(hexan-1-ol)-L-cysteine in *Vitis vinifera* L. cv. Koshu for aromatic wines. *Am. J. Enol. Vitic.*, 61, 176 - 185.

Lavigne V., Pons A., Darriet Ph., Dubourdieu D. - 2008 - Changes in the sotolon content of dry white wines during barrel and bottle aging. *J. Agric. Food Chem*., 56, 2688-2693.

LLedo P.M., Gheusi G., Vincent J.D. - 2004 - Information processing in the mammalian olfactory system. *Phys. rev.*, 85, 281-317.

Lopes P., Silva M., Pons A., Tominaga T., Lavigne V., Saucier C., Darriet Ph., Teissedre P.L., Dubourdieu D. - 2009 - Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. *J. Agric. Food Chem.,* 57, 10261-10270.

Marriott Ph., Eyres G., Dufour J.P. - 2009 - Emerging opportunities for flavor analysis through

hyphenated gas chromatography. *J. Agric. Food Chem.*, 57, 9962–9971.

Nikolantonaki N., Chichuc I.,Teissedre P.L., Darriet Ph. - 2010 - Reactivity of volatile thiols with polyphenols in a wine-model medium : Impact of oxygen, iron, and sulfur dioxide. *Anal. Chim. Acta*, 660, 102-109.

Pineau B., Barbe J.-C., Van Leeuwen C., Dubourdieu D. - 2009 - Examples of perceptive interactions involved in specific "red-" and "black-berry" aromas in red wines. *J. Agric. Food Chem.*, 57, 3702–3708.

Pons A., Lavigne V., Landais Y., Darriet Ph., Dubourdieu D. – 2008a - Distribution and organoleptic impact of sotolon enantiomers in dry white wines. *J. Agric. Food Chem.,* 56, 1606-1610.

Pons A., Lavigne V., Frérot E., Darriet Ph., Dubourdieu D. – 2008b - Identification of volatile compounds responsible for prune aroma in prematurely aged red wines. *J. Agric. Food Chem.,* 56, 5285–5290.

Pons A., Lavigne V., Landais Y., Darriet Ph., Dubourdieu D. - 2010 - Identification of a sotolon pathway in dry white wines. *J. Agric. Food Chem.*, 58, 7273-7279.

Roland A., Schneider R., Guernevé C.L., Razungles A., Cavelier F. - 2010 - Identification and quantification by LC-MS/MS of a new precursor of 3-mercaptohexan-1-ol using stable isotope dilution assay. Elements for understanding the 3MH production in wine. *Food Chem.,*121, 847-855.

Sarrazin E., Dubourdieu D., Darriet P. - 2007 - Characterization of key-aroma compounds of botrytized wines – Influence of grape botrytization. *Food Chem.,* 103, 536-545.

Sarrazin E. Shinkaruk S., Pons M., Thibon C., Bennetau B. Darriet P. - 2010 - Elucidation of 1,3-sulfanylalcohol oxidation mechanism: an unusual identification of the disulfide of 3-sulfanylhexanol in Sauternes botrytized wines. *J. Agric. Food Chem.,* 58.

Silva Ferreira A.C., Hogg T., Guedes de Pinho A. - 2003- Identification of key odorants related to the typical aroma of oxidation-spoiled wines. *J. Agric. Food Chem.,* 51, 1377-1381.

Shepherd G. - 2006 - Smell images and the flavour system in the human brain. *Nature,* 444, 316-321.

Thibon C., Dubourdieu D., Darriet P., Tominaga T. - 2009 - Impact of noble rot on the aroma precursor of 3-sulfanylhexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food Chem.*, 114, 1359-1364.