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Influence of volatile thiols in the development of blackcurrant aroma in red wine

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ABSTRACT

A strong blackcurrant aroma was recently perceived in some red wines originating from the same appellation. Varietal thiols such as 4-mercapto-4-methyl-2-pentanone (4MMP), 3-(mercapto)hexyl acetate (3MHA) and 3-mercapto-1-hexanol (3MH) are compounds potentially responsible for the development of this aroma. In order to demonstrate the correlation between thiols concentrations in red wines and blackcurrant aroma intensity, a multiple variable analysis was realised with thiols concentrations obtained by chemical analysis and blackcurrant aroma intensities obtained by descriptive sensory analysis. The 4MMP concentration was very well correlated to the blackcurrant aroma, and 3MHA and 3MH present at high concentrations act as enhancers of the perception of this aroma. This correlation was further supported after performing a sensory comparison by classification test. The different factors that could impact on the development of blackcurrant aroma in red wine were discussed.

1. Introduction

In recent years, a recurrent blackcurrant aroma has been described in red wines originating from the Languedoc region and, depending on its intensity, this aroma can be perceived as pleasant or unpleasant. However, the accidental occurrence of this aroma in certain red wines has caused problems when consumers give particular care to the local flavour. Several studies reported that the red-berry aroma in red wines resulted from the additive and synergistic effects of compounds from the same chemical family, such as ethyl esters and alkyl acetates, *via* perceptual interactions (Bouchilloux, Darriet, Henry, Lavigne-Cruège, & Dubourdieu, 1998; Fang & Qian 2005; Ferreira, Lopez, Escudero, & Cacho, 1998; Noguerol-Pato, González-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011; Pineau, Barbe, Van Leeuwen, & Dubourdieu 2009). A recent study on blackberry aroma in red wine reported the identification by gas chromatography/mass spectrometry of ethyl leucate in an active odour zone directly related to the blackberry flavour (Falcao, Lytra, Darriet, & Barbe, 2012). In the same study, a sensorial analysis showed the additive effect of ethyl leucate on blackcurrant flavour perception in red wine, but its direct impact on this aroma could not be demonstrated (Falcao et al., 2012).

However, it was demonstrated that the expression of the varietal thiol 4-mercapto-4 methyl-2-pentanone (4MMP) results in the development of blackcurrant aroma typical of Sauvignon white wines, and 4MMP could be responsible for such aroma development in red wines (Darriet, Lavigne, Boidron, & Dubourdieu, 1991; Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1993). Varietal thiols such as 4-mercapto-4-methyl-2-pentanone (4MMP), 3-(mercapto)hexyl acetate (3MHA) and 3-mercapto-1-hexanol (3MH), with perception thresholds in wine of 3 ng/L, 4 ng/L and 60 ng/L, respectively, are powerful odoriferous volatile compounds that constitute the varietal aroma of a wine. They result from the cleavage by *Saccharomyces cerevisiae* yeast of odourless cysteinylated and glutathionylated precursors present in grapes and must during alcoholic fermentation (Peyrot des Gachons, Tominaga, & Dubourdieu, 2002; Roland, Schneider, Guernevé, Razungles, & Cavelier, 2010; Roland, Vialaret, Moniatte, Rigou, Razungles, & Schneider, 2010; Tominaga, Peyrot des Gachons, & Dubourdieu, 1998).

The quantity of thiols present in white wines depends on the quantity of precursors present in grapes and must that varies with the grape variety, grape maturity at harvest, nitrogen and sulphur foliar supply and water deficit; on the pre-fermentation skin contact temperature; the yeast strain used during alcoholic fermentation; and on the oxidation reactions affecting the must during pre-fermentation stages and the thiols during alcoholic fermentation and wine ageing (Brajkovich et al., 2005; Howell et al., 2005; Roland, Schneider, Razungles & Cavelier, 2011).

Over the years, the presence of these compounds in wine was reported, but their role in the sensory characteristics of wine is not yet completely understood because of the difficulties involved in their analytical determination. These difficulties are related to the complexity of the wine matrix, the extremely low

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concentration levels at ng/L that must be determined, and the well-known instability and elusiveness of these compounds. In white wine, 4MMP is normally found at levels ranging from 1 to 60 ng/L, 3MHA at levels from 5 to 500 ng/L and 3MH at concentrations up to micrograms per litre. 4MMP was mainly identified in white wines such as Sauvignon Blanc, Colombard, Scheurebe, Muscat, and Petit Manseng, whereas 3MHA and 3MH are more ubiquitous and were identified in a wide range of white and red wines (Brajkovich et al., 2005; Darriet et al., 1993; Roland, Schneider, Razungles et al., 2011).

Various analytical methods were developed for the analysis of volatile thiols in wine at ppt levels, but methods based on derivatisation procedures appear to be the most powerful for such compounds (Roland, Schneider, Charrier et al., 2011). Chemical derivatisation of volatile thiols is an interesting method that was investigated using 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) as derivatising agent. In the case of low molecular weight volatile compounds present at trace levels in a complex matrix, this method yields stable derivatives and adds selectivity to the extraction method, with effective group separation of complex mixtures (Knapp, 1979). Regarding GC/MS analysis, derivatives of thiol compounds yield stabilised ions formed in the mass spectrometer and favour structurally informative fragmentations with high mass ions (Knapp, 1979).

An analytical method was optimised in our laboratory based on extraction of compounds on a solid-phase extraction (SPE) cartridge, derivatisation with PFBBr, concentration of compounds with solid-phase microextraction on a dry extract and analysis by GC/MS (Rodriguez-Bencomo, Schneider, Lepoutre, & Rigou, 2009). This method shows limits of quantification of 0.10, 0.83 and 4.3 ng/L for the analysis of 4MMP, 3MHA and 3MH, respectively, in white wine.

Using this method, 4MMP, 3MHA and 3MH were quantified in ten red wines originating from the Languedoc Roussillon region (France) and that may have typical blackcurrant aroma. These analyses were performed alongside descriptive sensory analysis. Using statistical methods, we tried to correlate varietal thiols concentrations and sensorial characterisation, in order to emphasise the contribution of one or several varietal thiols in the blackcurrant aroma of red wines. On the basis of the findings, the viticultural and oenological processes that may influence the development of blackcurrant aroma in wines were discussed.

2. Material and methods

2.1. Wine samples

This study was performed on ten red wines, whose characteristics are reported in Table 1, provided by local producers from Languedoc. These commercial wines were selected by eight winemakers not specifically trained to smell blackcurrant aroma but connoisseurs of this particular aroma, in order to provide wines with and without blackcurrant aroma. These wines were used both for chemical and sensory analysis.

2.2. Chemical analysis

2.2.1. Chemicals and reagents

Sulphur compounds 4MMP, 3MHA and 3MH (pure reference compounds) were obtained from Interchim (Montluçon, France); mercaptoglycerol (>98%), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (>99%), *o*-methylhydroxylamine hydrochloride (>99%) and L-cysteine hydrochloride monohydrate (>99%) from Fluka (Buchs, Switzerland); ethylenediaminetetraacetic acid disodium salt 2-hydrate, sodium chloride, anhydrous sodium sulphate and silica-gel from Merck (Darmstadt, Germany). Dichloromethane, methanol, diethyl ether, ethanol and hexane were obtained from Riedel de Häen (St. Quentin Fallavier, France), and 2,3,4,5,6-pentaflurobenzyl bromide (PFBBr) from Aldrich. The deuterated thiols (4MMP-d10, 3MH-d2 and 3MHA-d5) were synthesised within the laboratory [24].

Bond Elut-ENV cartridges of 500 mg were from Varian (Walnut Creek, CA), Visiprep SPE vacuum manifold and Didvinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (50/30 μm; 2 cm length) were from Supelco (Bellefonte, PA).

2.2.2. Extraction of thiols from wine

Extractions and analysis of thiols from red wines were performed using the stable isotope dilution assay (SIDA) method published by others (Rodriguez-Bencomo et al., 2009). Deuterated standards were all synthetised in our laboratory. The extraction method is based on the methoximation of the 4MMP directly in the wine, followed by solid-phase extraction of the thiol compounds on a Bond Elut-ENV cartridge and the derivatisation of all thiols with pentafluorobenzyl bromide directly on the cartridge. Derivatised thiols are then eluted with 4 mL of hexane/diethyl ether (1:3). This extract was then evaporated to dryness in a 10-mL SPME vial that was then sealed under nitrogen. It was then extracted for 30 min using an SPME fibre (DVB/CAR/PDMS) at 110 °C using an automatic CombiPal system (CTC Analytics, Zwingen, Switzerland). Compounds were directly desorbed from the fibre in the GC injector in splitless mode for 10 min at 250 °C.

2.2.3. GC–MS analysis

Chromatographic analyses were carried out using a Shimadzu QP5050 GC–MS coupled to a quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The capillary column was an Optima-Wax (30 m × 0.25 mm × 0.25 μm) from Macherey-Nagel GmbH & Co. KG, Düren, Germany). The temperature program of the column oven was: 80 °C for 10 min, heated to 220 °C at 5 °C min^{-1} and to

Table 1
Characteristics of the wines used for the study.

Wine	Appellation of origin	Cultivar	Age	Type of yeast used
769	AOC Coteaux du Languedoc	Syrah 80%/Grenache 10%/ Mourvedre 10%	2007	Exogenous
102	AOC Coteaux du Languedoc	Syrah 80%/Grenache 10%/ Mourvedre 10%	2009	Endogenous
203	AOC Coteaux du Languedoc	Grenache 65%/Mourvedre 35%	2008	Exogenous
052	AOC Coteaux duLanguedoc	30% Grenache/30%Syrah/ 20%Cinsault/20% Carignan	2008	Endogenous
839	AOC Coteaux du Languedoc	40% Grenache/30% Syrah/ 10%Carignan/10% Cinsault/ 10% Mourvedre	2009	Endogenous
415	AOC Coteaux du Languedoc	65% Grenache/15%Carignan/ 15% mourvedre/5% Cinsault	2009	Exogenous
328	AOC Coteaux du Languedoc	45% Syrah/35% Grenache/20% Mourvèdre	2010	Exogenous
618	AOC Coteaux du Languedoc	80% Syrah/20%Grenache	2009	Exogenous
467	AOC Coteaux du Languedoc	30% Syrah/ 30% Grenache/20% Cinsault/20% Carignan	2010	Exogenous
577	AOC Coteaux du Languedoc	30% Syrah/50% Mourvèdre/ 20% Grenache noir	2006	Exogenous