Study of the influence of varietal amino acid profiles on the polyfunctional mercaptans released from their precursors

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ABSTRACT

Polyfunctional mercaptans such as 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexanol and 3-mercaptohexyl acetate contribute strongly to the varietal aroma of wines. These compounds are released during alcoholic fermentation from their precursors and their concentration is strongly linked to the grape variety. The aim of this work was to determine the effect of nine different amino acid profiles of grape varieties on the release of these polyfunctional mercaptans and on the consumption of their precursors as well as to determine which are the preferred precursors. A set-up of fermentations of synthetic must was prepared, which simulated the amino acid profiles of nine different grape varieties, containing known amounts of polyfunctional mercaptan precursors as well as the elements necessary for the yeast metabolism. The yeast assimilable nitrogen was adjusted in percentage to reach 150 mg N/L in all the fermentations. Polyfunctional mercaptans and their precursors were analyzed in the final wine by gas chromatography coupled to mass spectrometry with negative chemical ionization and ultrahigh liquid performance chromatography coupled to mass spectrometry, respectively. The results showed significant differences in the release of these polyfunctional mercaptans as well as in the consumption of their precursors according to the amino acid profile. Moreover, it was observed that the preferred precursor of 4-mercapto-4-methyl-2-pentanone was its cysteinylated precursor. These results suggest that the amount of the amino acids as well as the ratio between them could modify the amount of polyfunctional mercaptans released. This could be a tool for the wine industry to vary the aromatic profile of wines by increasing or decreasing these volatile thiols.

1. Introduction

Sulfur compounds, especially polyfunctional mercaptans, may play an important role in the aroma of many products, both fresh (plants, vegetables, fruit, etc.) and processed (roasted coffee, wine, etc.) (Harsch & Gardner, 2013; Tominaga, Murat, & Dubourdieu, 1998). Polyfunctional mercaptans are varietal aroma compounds that contribute to the aroma characteristics of white and rosé wines, and also in some red wines (Harsch & Gardner, 2013; Roland, Schneider, Razungles, & Cavelier, 2011). The main aromatic polyfunctional mercaptans are 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) associated with notes of grapefruit, citrus zest, passion fruit, gooseberry and guava, and 4-mercapto-4-methyl-pentan-2-one (4MMP), typically characterized as boxwood, blackcurrant, broom and passion fruit aromas (Lund et al., 2009; Mestres, Busto, & Guasch, 2000; Swiegers & Pretorius, 2007; Tominaga, Murat, & Dubourdieu, 1998). These compounds are considered to have a key impact, since they are often found in concentrations far above their olfactory perception thresholds, 60.4 ng/L for 3MH, 4 ng/L for 3MHA and 0.8 ng/L for 4MMP (Tominaga, Murat, & Dubourdieu, 1998). 4MMP was first identified in Sauvignon Blanc (SB) wine (Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1995). 3MH and 3MHA have been identified in a wide range of varietal wines (Campo, Ferreira, Escudero, & Cacho, 2005; Tominaga, Baltenweck-Guyot, des Gachons, & Dubourdieu, 2000; Tominaga, Darriet, & Dubourdieu, 1996). These studies suggest that polyfunctional mercaptans have an important role in the aroma of wines from different grape varieties. Nevertheless, the influence of the variety like amino acid profile is not clear.

3MH and 4MMP are present in grapes linked with a molecule of cysteine (Tominaga, des Gachons, & Dubourdieu, 1998) or glutathione (des Gachons, Tominaga, & Dubourdieu, 2002; Fedrizzi, Pardon, Sefton, Elsey, & Jeffery, 2009) forming the following odorless precursors: glutathione-3-mercaptohexan-1-ol (GLUMH), glutathione-4-mercapto-4-methyl-2-pentanone (GLUMP), cysteine-3-mercaptohexan-1-ol (CYSMH) and cysteine-4-mercapto-4-methyl-2-pentanone (CYSMP). Furthermore, in 2002, des Gachons et al. demonstrated that the

Table 1
Composition (in milligrams per liter) of amino acids related to 7 grape varieties-like profiles and 2 versions of one of these varieties adjusted to reach 150 mg/L of total nitrogen.

	Aspartic acid	Glutamic acid	Serine	Glycine	Histidine	Threonine	Arginine	Proline	Methionine	Phenylalanine	Lysine	Glutamine	GABA
Cabernet Sauvignon-like profile	15.6	32.8	24.6	2.91	221	27.9	56.0	1207	30.7	3.94	0.00	50.3	35.1
Grenache-like profile	26.7	47.5	16.3	0.85	83.9	14.4	153	185	22.9	11.2	2.56	80.5	38.4
Tempranillo-like profile	27.7	27.3	19.3	2.07	44.0	23.2	215	96.9	8.08	2.41	4.39	56.8	16.0
Chardonnay-like profile	33.6	98.1	74.0	2.58	33.9	56.4	137	360	9.21	24.4	8.38	72.3	43.0
Carignan-like profile	29.0	96.2	34.9	0.00	68.0	27.6	154	539	14.3	5.54	0.85	54.6	50.0
Macabeo-like profile	31.7	31.8	25.4	3.71	55.3	22.7	143	126	17.8	13.3	0.00	142	22.0
SB-like profile	22.6	76.6	32.7	1.14	18.6	31.5	204	160	4.23	16.4	5.44	66.6	22.9
SBv1-like profile	20.8	70.5	30.1	1.05	17.1	29.0	188	147	3.89	15.1	5.00	61.3	84.1
SBv2-like profile	13.7	186	19.8	0.69	11.3	19.1	124	97.6	2.57	9.98	3.30	161	55.5

enzymatic conversion of GLUMH into CYSMH is possible (des Gachons et al., 2002).

Aromatic thiols are released from their precursors during alcoholic fermentation (AF) when the β-lyase enzymatic action of yeast produces the cleavage of the carbon-sulfur bond (Tominaga, des Gachons, & Dubourdieu, 1998). The synthesis of 3MHA during must fermentation consists of the acetylation of the volatile thiol 3MH by the action of the yeast alcohol acetyltransferase (Swiegers & Pretorius, 2007; Swiegers et al., 2006). However, there is no direct correlation between precursor concentrations in grape musts and thiol concentrations in the final wines since only a small percentage of the precursors seem to be converted into the corresponding volatile thiol (des Gachons, 2000). Moreover, to the best of our knowledge, the ability of yeasts to use each of the precursors during AF, particularly 4MMP precursors, has not been studied.

The abundance of precursors seems to be linked to the grape variety (Coetzee & du Toit, 2012) and yeast assimilable nitrogen (YAN) (Chone et al., 2006). In addition, YAN is also very important for the yeast growth and fermentation metabolism. The main sources of YAN in grape juice are ammonium ions and amino acids (Henschke & Jiranek, 1993). During alcoholic fermentation, yeast strains can use these nitrogen sources in several ways, particularly for protein synthesis and growth (Bauer & Pretorius, 2000; Styger, Prior, & Bauer, 2011; Vilanova et al., 2007). Therefore, supplementation with amino acids is an alternative approach but this can modulate the aroma profile of the final wine (Hernández-Orte, Ibarz, Cacho, & Ferreira, 2006). The amino acid profile may affect the order in which different amino acids are used by yeast, and it may also affect the production of volatile compounds (Hernández-Orte, Cacho, & Ferreira, 2002).

The aim of this work is to test whether the different amino acid profiles influence the use of polyfunctional mercaptan precursors by yeast during AF, as well as to identify the precursors preferred by the yeast. For this purpose, synthetic media with different amino acid profiles simulating different grape varieties were fermented. Likewise, fermentations of model solutions with precursors added separately with different amounts of YAN were also carried out. At the end of the AF, the precursors and polyfunctional mercaptans were determined and the correlation between them studied.

2. Materials and methods

2.1. Chemicals and reagents

n-Hexane for organic trace analysis (UniSolv), gradient grade ethanol for liquid chromatography (LiChrosolv), and diethyl ether (EMSURE) were supplied by Merck (Darmstadt, Germany) while high-performance liquid chromatography (HPLC) quality dichloromethane and methanol were obtained from Fisher Scientific (Loughborough, UK). Anhydrous sodium sulfate of analysis ACS-ISO quality was purchased from Panreac (Barcelona, Spain). Ethylenediaminetetraacetic acid disodium salt 2-hydrate (EDTA), L-cysteine hydrochloride hydrate

99%, 1,4-dithioerythritol, octafluoronaphthalene 96% (OFN), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were supplied by Aldrich (Steinheim, Germany). O-methylhydroxylamine hydrochloride purum > 98% and 2,3,4,5,6-pentafluorobenzylbromide (PFBBr) were obtained from Fluka (Buchs, Switzerland). 4MMP 98% and 3MH > 95% were purchased from Alfa Aesar (Karlsruhe, Germany). 3MHA was obtained from Oxford Chemical (Hartlepool, U.K.). 4-methoxy-α-toluenethiol was supplied by Fluka. Pure standards of the four precursors CYSMP, GLUMP, CYSMH and GLUMH were synthesized by Roowin (Riom, France), having a purity ≥ 95%. Bond Elut-ENV resins, prepacked in a 50 mg cartridge (1 mL total volume) and a semiautomated solid-phase extraction (SPE) Vac Elut 20 station, were supplied by Varian (Walnut Creek, CA). Liquid chromatography coupled to mass spectrometry (LC–MS) grade acetonitrile and formic acid obtained from Scharlau (Barcelona, Spain) were used as mobile phases.

2.2. Culture conditions

2.2.1. Synthetic medium

The synthetic medium used contained 105 g/L of glucose and the same amount of fructose. This medium (pH 3.5) was adapted from that described by Bely et al. (Bely, Sablayrolles, & Barre, 1990) with the following modifications: i) 150 mg N/L as ammoniacal nitrogen $((NH_4)_2HPO_4)$ 114.6 mg/L, amino acids with fixed concentrations (expressed in mg/L): L-alanine 60.1, L-leucine 20.2, L-valine 20.1, L-isoleucine 20.2, L-tyrosine 20.3, L-cysteine 5.07 and amino acids with variable concentrations to simulate amino acid profiles of different grape varieties (Table 1); and ii) 120 mg N/L with the same ammoniacal nitrogen and amino acids with the same fixed concentrations as mentioned above, whereas the amino acids with variable concentrations were obtained by adjusting the percentages of the amino acid profile of the Chardonnay-like profile (Table 1).

2.2.2. Amino acid profiles

The amino acid profiles of seven different grape varieties (Sauvignon Blanc (SB), Chardonnay, Macabeo, Carignan, Grenache, Tempranillo and Cabernet Sauvignon) were selected (Hernández-Orte et al., 2002). The amino acid profile of the SB-like profile was modified twice, SB version 1 (SBv1) and SB version 2 (SBv2), through the modification of three amino acids (γ-aminobutyric acid (GABA), glutamic acid and glutamine) based on the previous study of Pinu et al. concerning the contribution of these nitrogenous compounds to volatile thiol development (Pinu et al., 2014).

2.2.3. Precursors

The odorless precursors, CYSMH, CYSMP, GLUMH and GLUMP were added to a synthetic must in concentrations of 50 μg/L for CYSMP and GLUMP, 100 μg/L in the case of CYSMH and 1000 μg/L for GLUMH. Moreover, the cysteinylated precursors (with the same aforementioned concentrations) were added to a synthetic must of Chardonnay-like profile which had 120 and 150 mg N/L, and the same