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Factors influencing the formation of histaminol, hydroxytyrosol, tyrosol, and tryptophol in wine: Temperature, alcoholic degree, and amino acids concentration

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ABSTRACT

The validation of a HPLC–PDA–MS/MS chromatographic method for the quali/quantitative characterization of histaminol, hydroxytyrosol, tyrosol, and tryptophol in wine has been described and discussed. Four standards showed a good linearity with high correlation coefficient values (over 0.9989) and LOD and LOQ were 0.001–0.015 mg/L and 0.004–0.045 mg/L, respectively. Furthermore, this study reported how factors such as temperature, alcoholic degree, and amino acids concentration are able to influence the formation of these four alcohols in Monastrell wines. The quantification values of these alcohols has been detected both at the half and end of alcoholic fermentation, and at the end of malolactic fermentation. In relation to interactions between factors, several significant variations emerged ($p \leqslant 0.001$). The impact of amino acids supplementation in Monastrell must it has been demonstrated, mainly in regards to histaminol and tryptophol.

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1. Introduction

The conversion of amino acids is an important topic in the field of fermented foods. Amino acids together with proteins and peptides play an important role as nitrogen sources for yeast and lactic acid bacteria, respectively, during alcoholic and malolactic fermentations (Garde-Cerdán et al., 2009; Moreno-Arribas & Polo, 2009; Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). The importance of nitrogen for growth and fermentation activity is well-established (Arias-Gil, Garde-Cerdán, & Ancín-Azpilicueta, 2007; Bisson, 1999; Garde-Cerdán et al., 2011). The amount of nitrogen in must influences the wine quality (Carrau, Medina, Farina, Boido, & Dellacassa, 2010; Garde-Cerdán & Ancín-Azpilicueta, 2008; Martínez-Gil et al., 2012; Torrea et al., 2011). Nitrogen deficiency, commonly observed in many viticultural regions, is considered a growth-limiting factor (Bell & Henschke, 2005). Low nitrogen in grape must leads to low yeast populations and poor fermentation vigor. This adverse situation increased risk of sluggish or stuck fermentations, and the production of undesirable compounds (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009; Ugliano, Siebert, Mercurio, Capone, & Henschke, 2008; Vilanova et al., 2007). Biogenic amines and alcohols represent the two main molecular groups through which the starting amino acids are converted. Biogenic amines are produced by microbial decarboxylation of the corresponding amino acid precursors (López et al., 2011). These bioactive compounds in wine play an important role, not only because an intake of high levels of biogenic amines can lead to a variety of symptoms in humans, but these molecules may cause spoilage in wine (Smit & du Toit, 2013). The other catabolic way adopted by microorganisms produces alcohols (fusel alcohols), which derive from amino acids through the well-known Ehrlich pathway (Eden, Van Nedervelde, Drukker, Benvenisty, & Debourg, 2001). Yeasts convert amino acids through three enzymatic steps: transamination to form α-keto acid, decarboxylation to an aldehyde, and reduction to the fusel alcohol (Dickinson, Salgado, & Hewlins, 2003; Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008).

It is known that *Saccharomyces cerevisiae* can use tyrosine and tryptophan as the only source of cellular nitrogen, the main

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products of their catabolism being respectively tyrosol, hydroxytyrosol, or tryptophol (Garde-Cerdán & Ancín-Azpilicueta, 2008; Hazelwood et al., 2008). These represent compounds of pharmaceutical interest showing several health-enhancing activities, deriving from their free radical scavenging, anticarcinogenic, cardiopreventive and antimicrobial properties (Covas et al., 2003; Kris-Etherton et al., 2002). However, concerning a well-known fermented product such as wine, the analysis of the corresponding alcohol derived from catabolism of L-histidine (histaminol) has not been extensively studied even though its presence in wine has been detected by our previous study conducted on a set of several commercial wines (Bordiga, Travaglia, Locatelli, Arlorio, & Coïsson, 2010). According to the Ehrlich pathway, L-histidine should be transformed by yeast into a higher alcohol, in this case histaminol. However, a study focused on describing the characteristics of this process is not reported in literature. Based on the assumptions described previously, the first purpose of this work was to synthesize both the histaminol and hydroxytyrosol standard molecules and subsequently to develop and validate a HPLC–PDA–MS/MS chromatographic method for a quali/quantitative characterization of these two molecules, also including tyrosol and tryptophol. The second purpose was to evaluate how different factors such as temperature, alcoholic degree, and amino acids concentration/supplementation could have influenced the formation of these four alcohols (histaminol, hydroxytyrosol, tyrosol, and tryptophol) in wine.

2. Materials and methods

2.1. Chemicals

Chromatographic solvents were of HPLC–MS grade and were purchased from Sigma–Aldrich (Milan, Italy). Water was obtained by Milli-Q instrument (Millipore Corp., Bedford, MA). Standards of tyrosol (purity > 99.5%) and tryptophol (purity > 97%) were purchased from Sigma–Aldrich.

2.2. Synthesis and characterization of histaminol and hydroxytyrosol

2.2.1. Histaminol

The purified histaminol ($C_5H_8N_2O$; 112.2 g/mol), synthesized as previously reported by Bordiga et al. (2010), was characterized by means of ^{1}H NMR (CD_3OD, 300 MHz, 298 K), ^{13}C NMR (CD_3OD, 75.4 MHz, 298 K) and mass analysis (ESI/MS (+): m/z 113 $[M-H]^+$). Purity assessed by ^{1}H NMR (99.25 ± 0.25%) was determined considering the signals of residual solvent.

2.2.2. Hydroxytyrosol

3,4-Dihydroxyphenylacetic acid (400 mg, 2.38 mmol) was dissolved in anhydrous THF (15 mL), and then lithium aluminum hydride (361 mg, 9.52 mmol) was added. The mixture, after three vacuum-nitrogen cycles, was stirred under hot refluxing for 90 min.

Then, the mixture was acidified with 2 M H_2SO_4 and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were dried, filtered and take to dryness. The residue was purified by gravity column chromatography using CH_2Cl_2/acetone 80:20 (v/v) to obtain hydroxytyrosol (light yellow oil, 348 mg, 95% yield). The purified hydroxytyrosol ($C_8H_{10}O_3$; 154.1 g/mol) was characterized by means of ^{1}H NMR (acetone-d_6, 400 MHz, 298 K), ^{13}C NMR (acetone-d_6, 100.6 MHz, 298 K) and mass analysis (ESI/MS (+): m/z 155 $[M-H]^+$) as reported in Figure S1 (supplementary material). Purity assessed by ^{1}H NMR (98.50 ± 0.25%) was determined considering the signals of residual solvent.

Table 1
Oenological parameters of the different wines at the end of alcoholic fermentation.

Temperature	Alcohol	Added amino acids	Alcohol (%, v/v)	pH	Total acidity (g/L)	Volatile acidity (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Color intensity	Hue	TPI	Anthocyanins (mg/L)
22 °C	Normal	0	12.5 ± 0.3	3.4 ± 0.0	6.1 ± 0.1	0.26 ± 0.06	2.0 ± 0.1	0.26 ± 0.01	5.6 ± 0.2	0.51 ± 0.02	3.5 ± 0.1	222 ± 16
		His	12.84 ± 0.08	3.4 ± 0.0	6.01 ± 0.08	0.24 ± 0.01	2.04 ± 0.03	0.21 ± 0.01	5.59 ± 0.04	0.51 ± 0.00	3.52 ± 0.01	217.9 ± 0.3
		His + Tyr + Trp	12.7 ± 0.2	3.3 ± 0.0	6.1 ± 0.0	0.24 ± 0.01	2.04 ± 0.04	0.26 ± 0.01	5.7 ± 0.0	0.51 ± 0.00	3.6 ± 0.0	220 ± 7
22 °C	+3	0	15.42 ± 0.09	3.50 ± 0.01	5.8 ± 0.1	0.45 ± 0.03	2.01 ± 0.03	0.27 ± 0.03	5.55 ± 0.05	0.55 ± 0.01	3.64 ± 0.08	206 ± 7
		His	15.49 ± 0.01	3.50 ± 0.01	5.86 ± 0.08	0.43 ± 0.01	2.04 ± 0.01	0.25 ± 0.01	5.55 ± 0.06	0.56 ± 0.00	3.59 ± 0.01	206 ± 5
		His + Tyr + Trp	15.7 ± 0.1	3.51 ± 0.01	5.77 ± 0.02	0.45 ± 0.01	1.95 ± 0.04	0.25 ± 0.01	5.45 ± 0.04	0.57 ± 0.01	3.64 ± 0.06	198 ± 3
16 °C	Normal	0	12.7 ± 0.1	3.37 ± 0.01	6.06 ± 0.06	0.28 ± 0.00	2.24 ± 0.06	0.18 ± 0.02	5.42 ± 0.03	0.50 ± 0.00	3.45 ± 0.03	216 ± 0
		His	12.70 ± 0.08	3.37 ± 0.01	6.0 ± 0.1	0.29 ± 0.01	2.16 ± 0.07	0.22 ± 0.01	5.39 ± 0.00	0.51 ± 0.00	3.44 ± 0.04	213.35 ± 0.07
		His + Tyr + Trp	12.86 ± 0.03	3.37 ± 0.01	5.83 ± 0.01	0.29 ± 0.01	2.09 ± 0.04	0.24 ± 0.02	5.32 ± 0.04	0.52 ± 0.01	3.54 ± 0.05	208 ± 1
16 °C	+3	0	15.4 ± 0.2	3.51 ± 0.01	5.5 ± 0.1	0.48 ± 0.01	1.98 ± 0.07	0.25 ± 0.04	5.36 ± 0.08	0.56 ± 0.01	3.53 ± 0.01	200 ± 3
		His	15.4 ± 0.3	3.51 ± 0.01	5.56 ± 0.1	0.46 ± 0.01	1.99 ± 0.08	0.22 ± 0.02	5.35 ± 0.08	0.57 ± 0.01	3.51 ± 0.01	198 ± 5
		His + Tyr + Trp	15.33 ± 0.07	3.54 ± 0.00	5.79 ± 0.03	0.52 ± 0.01	2.16 ± 0.05	0.24 ± 0.01	5.22 ± 0.00	0.56 ± 0.00	3.50 ± 0.04	193.2 ± 0.4

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