AROMA PRECURSORS OF GRAPES: CONTRIBUTION OF VARIETY AND VINEYARD TRANING SYSTEM TO PORT WINE AROMA

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ABSTRACT

In order to evaluate the influence of the vine training system in the composition in terpenes, norisoprenoids and their precursors (carotenoids and glycosidic molecules), these compounds were quantified in grapes and Port wines belonging to two varieties: Touriga Nacional and Touriga Franca from Douro Region. The double cordon LYS 2/3 system was compared with the traditional method, the vertical shoot positioning VSP system. Touriga Nacional grapes and wines have higher contents in carotenoids, free and glycosylated fractions of terpenic and norisoprenoids compounds than Touriga Franca. In general, LYS 2/3 training system produced grapes and wines with high content in glycosylated fractions of terpenes and nor-isoprenoids, being more appropriate to the production of Port wines, which are submitted to an aging process.

RESUMO

A composição em terpenos e norisoprenoides, fracção livre e ligada, bem como os seus precursores, foi avaliada em uvas e vinhos do Porto provenientes das castas Touriga Nacional e Touriga Franca da Região do Douro. Dois sistemas de condução, LYS 2/3 (duplo cordão) e VSP (sistema tradicional) foram estudados. As uvas e vinhos provenientes da Touriga Nacional apresentaram uma maior concentração em carotenoides, bem como terpênos e norisoprenoides livres e ligados que a Touriga Franca. Em geral, o sistema LYS 2/3 produziu uvas e vinhos com teores em terpenos e norisoprenoides ligados superiores ao sistema tradicional, sendo o sistema mais apropriado para a produção de vinhos do Porto que vão ser sujeitos a envelhecimento.

Introduction

Terpenes and norisoprenoids are responsible for the varietal aroma of wines. Linalol, geraniol, nerol, α-terpineol, citronellol and HO-trienol are the most odoriferous terpenic alcohols, which contribute to the floral aroma of wines (Mateo, Jimenez, 2000; Maicas, Mateo, 2005). The olfactory perception thresholds of these compounds are rather low, as little as a few micrograms per liter. Terpenes are present in grapes, in free and glycosylated form; the terpene contents increase during alcoholic fermentation due to the β-glucosidase activity of yeasts. Norisoprenoids could be produced from direct carotenoid molecules degradation, such as β-carotene, lutein, neoxanthin and violaxanthin (Winterhalter, 1991) and from the hydrolysis of glycoside molecules (Mendes Pinto, 2003; Silva Ferreira, Guedes de Pinho, 2004; Cabrita *et al*., 2006; Oliveira *et al*., 2006). Norisoprenoids compounds contribute to fruity, floral and spicy notes, and play a high sensory impact on wine aroma, especially in *Chardonnay*, *Semillon*, *Sauvignon blanc*, *Riesling* and *Chenin blanc* wines (Winterhalter, Rouseff, 2002). Some norisoprenoids has been identified in wines, namely β-damascenone, β-ionone, vitispirane, 1,1,6-trimethyl-1,2-dihydronaohtalene (TDN), 2,2,6-trimethylcyclohexanone (TCH), and more recently (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) (Janusz *et al*., 2003; Silva Ferreira, Guedes de Pinho, 2004; Ribéreau-Gayon *et al*., 2005; Oliveira *et al*., 2006; Silva Ferreira *et al*., 2008).

In order to understand the effect of the vineyard training system on the composition of Port wines, in terms of carotenoids and, free and bounded fractions of terpenes and norisoprenoids, experiments were carried out using grapes from Touriga Nacional and Touriga Franca varieties. The double cordon LYS 2/3 system was compared with the traditional method, the vertical shoot positioning VSP system.

Material and methods

Grapes and fermentation conditions

Experiments were carried out with grapes of the *Vitis vinifera* varieties of the Douro Region: Touriga Nacional and Touriga Franca. After harvest, the grapes were crushed, pressed and treated with sulphite solution to achieve 50 mg/L of SO_2. The grape musts were transferred to stainless steel tanks of 20L. All experiments were carried out in duplicate. Musts were inoculated with a rehydrated culture of *Saccharomyces cerevisiae* var. *bayanus* (commercial name C1108® from Proenol, Lallemand), at 30 g/hL. All wines were produced using the same vinification procedure. Fermentations were carried out at 18 ºC, during 3 days. At this time, grape spirit was added, to stop fermentation, and to obtain an alcoholic degree of 19% (vol.). The skins and seed were removed by dejuicing and pressing.

Analytical determinations

Carotenoids and chlorophyll-derived compounds were analysed by HPLC-DAD in grape according to the method described by Mendes Pinto *et al*. (2005). Free and glycosidic fractions of norisoprenoids and terpenes in grapes and Port wines were analysed by GC-MS (Oliveira *et al*., 2006).

Results and discussion

The profile of carotenoids of Touriga Nacional and Touriga Franca grapes allowed the identification of 11 compounds with carotenoid structure and two compounds derived from chlorophyll (Fig. 1). Lutein, pheophytin b and β-carotene were the higher compounds found in grapes, which corroborate some studies described in literature (Razungles *et al*., 1988; Guedes de Pinho *et al*., 2001, 2007; Baumes *et al*., 2002; Mendes Pinto, 2003; Oliveira *et*

al., 2006; Mendes Pinto *et al*., 2005). Only the β-carotene, lutein, neoxanthin, violaxanthin and luteoxanthin compounds were quantified.

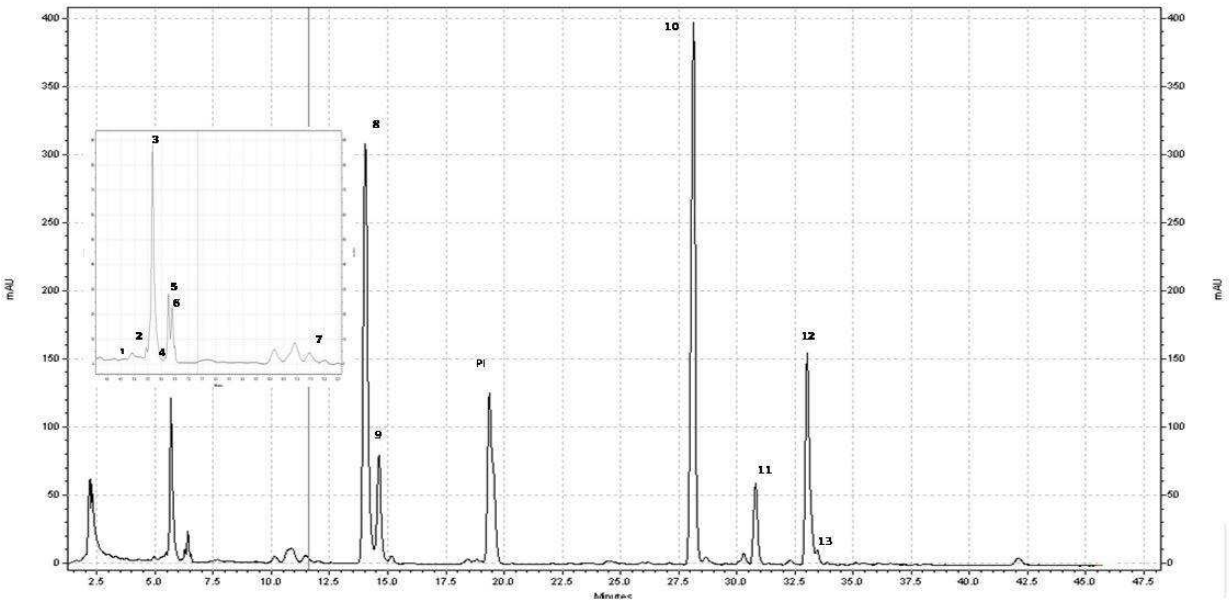

Fig. 1. Separation by reverse phase HPLC of carotenoids in Touriga Nacional grapes for VSP system: (1) peak 1^{A}, (2) neocromo A, (3) neocromo B, (4) neoxanthin, (5) violaxanthin, (6) luteoxanthin, (7) peak 2^{A}, (8) lutein, (9) peak 3^{A} (structure similar to lutein), (10) pheophytin b, (11) pheophytin a, (12) β-carotene, (13) (Z)-β-carotene. ^{A} unidentified compounds.

The grapes from Touriga Nacional variety presented higher content in carotenoids than Touriga Franca grapes (Table 1). For both training systems, the carotenoid content was similar (1267 and 1258 μg/Kg). In Touriga Franca grapes, VSP system allowed a high carotenoid content (903 μg/Kg) than LYS 2/3 system (717 μg/Kg). For both varieties and vineyard training systems, the carotenoid contents are in accordance with the ranged values referenced in the literature (800 μg/kg and 2500 μg/kg) (Razungles *et al*., 1987).

Although 16 terpene compounds and 10 norisoprenoids identified, only linalol, nerol, α-terpineol, 4-terpineol, HO-trienol, α-terpinene, α-terpinolene, β-damascenone, β-ionone, TDN and TPB were quantified.

Table 1. Carotenoids content of Touriga Nacional and Touriga Franca grapes in VSP and LYS 2/3 training systems.

Compound (μg/Kg)	Touriga Nacional		Touriga Franca	
	VSP	LYS 2/3	VSP	LYS 2/3
Neoxanthin	nd	61.1	63.3	50.8
Violanthin	12.1	18.6	2.4	6.5
Luteoxanthin	2.4	16.1	3.9	6.0
Lutein	516	424	175	154
β-Carotene	737	739	658	500
Total carotenoids	1267	1259	903	717

nd – not detected

Touriga Nacional grapes presented a concentration in free terpene compounds higher in VSP system (28.3 μg/kg) than in LYS 2/3 (14.3 μg/kg). In Touriga Franca grapes, the free fraction of terpenic compounds was only observed in LYS 2/3 system (data not shown), being the norisoprenoids only present in grapes in their glycosylated form (Ribéreau-Gayon *et al*., 2005).

The concentrations of terpene compounds in the glycosylated fraction of Touriga Nacional and Touriga Franca grapes are shown in Fig. 2. In both vineyard training systems, the highest amounts of terpene compounds in the glycosylated fraction were obtained in Touriga Nacional grapes. The VSP system produced grapes with lower amounts of these compounds than LYS 2/3 system.

The glycosylated fraction of norisoprenoids in Touriga Nacional and Touriga Franca grapes is represented in Fig. 3. As expected, Touriga Nacional showed higher contents of norisoprenoids in the glycosylated fraction than Touriga Franca. Despite the similar contents in norisoprenoids for both training systems, it was grapes from LYS 2/3 system that presented the highest amounts of β-damascenone (2.79 μg/kg) and β-ionone (0.80 μg/kg), whereas the VSP system presented highest amounts of TDN (4.63 μg/kg) and TPB (1.27 μg/kg).

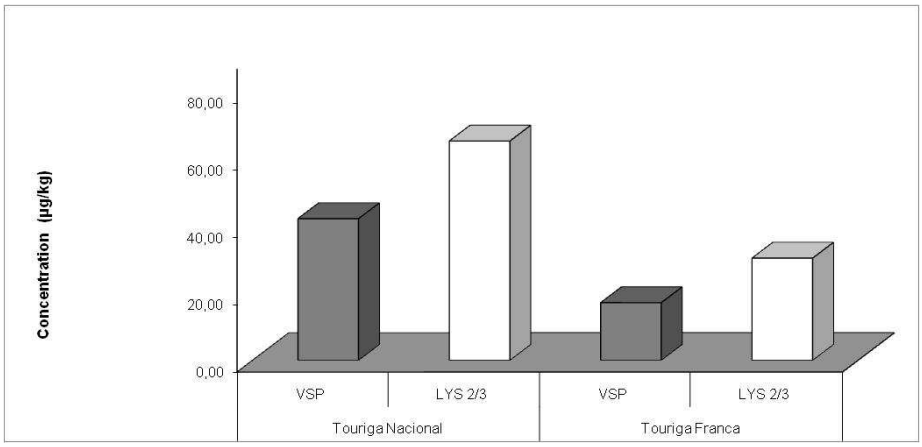

Fig 2. Concentration of terpenic compounds in the glycosylated fraction of Touriga Nacional and Touriga Franca grapes, in VSP and LYS 2/3 training systems.

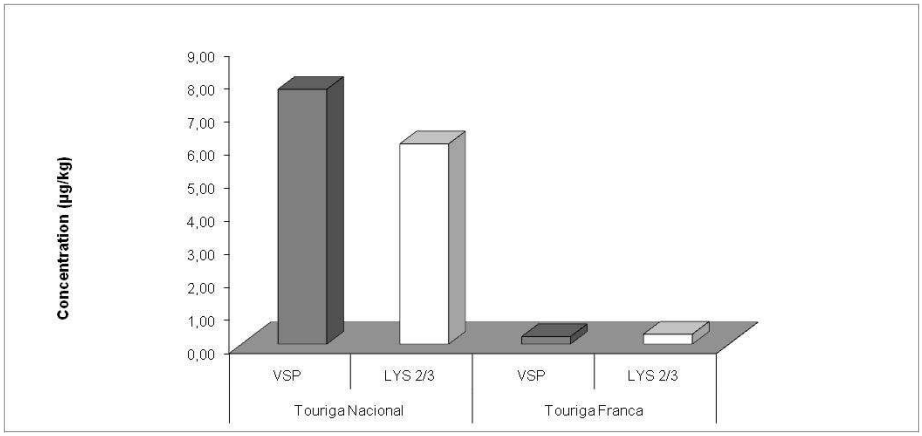

Fig. 3. Total of norisoprenoids in the glycosylated fraction of Touriga Nacional and Touriga Franca grapes, in VSP and LYS 2/3 systems.

Although its low aromatic potential, Touriga Franca has other characteristics (higher phenolic compound, great yielding), that make this variety as an excellent variety to use in Port wine.

The contents of different terpenic compounds present in the free and bounded fraction of wines from both varieties and, for two vineyard training systems, are presented in Table 2. Touriga Nacional wines showed a higher content of free terpenic compounds than Touriga Franca wines. Similar results were obtained by Oliveira *et al*. (2006). Touriga

Nacional wines from VSP system presented a high levels in the free terpenes than those obtained from LYS 2/3 system; however, the vineyard conducted system had no effect in the content of free terpenes of Touriga Franca wines.

Table 2. Concentration of free and glycosylated terpenes in Touriga Nacional and Touriga Franca wines for VSP and LYS 2/3 vineyard training systems.

Compound (μg/L)	Touriga Nacional		Touriga Franca	
	VSP	LYS 2/3	VSP	LYS 2/3
Free terpenes	317	145	25	23
Glycosylated terpenes	862	1128	201	134

As expected, glycosylated terpenic compounds in Touriga Franca were lower than those obtained in Touriga Nacional, for both vineyards training systems. Concerning Touriga Nacional wines, LYS 2/3 system showed the highest contents. Linalol, nerol and α-terpineol, were present in Touriga Nacional wines above its perception threshold, being responsible for varietal wine aroma.

Concerning free norisoprenoids, Touriga Nacional wines showed the highest content in LYS 2/3 system (data not shown). Only β-damascenone and β-ionone were identified in Touriga Nacional wines. As expected, Touriga Nacional wines showed higher content in glycosylated norisoprenoids than Touriga Franca. Port wines obtained from LYS 2/3 system, showed higher composition in glycosylated norisoprenoids than those obtained from VSP system (Fig. 4). Among all norisoprenoids determined, Touriga Nacional LYS 2/3 system showed higher amounts in β-damascenona and β-ionona which contribute to the varietal aroma of these wines.

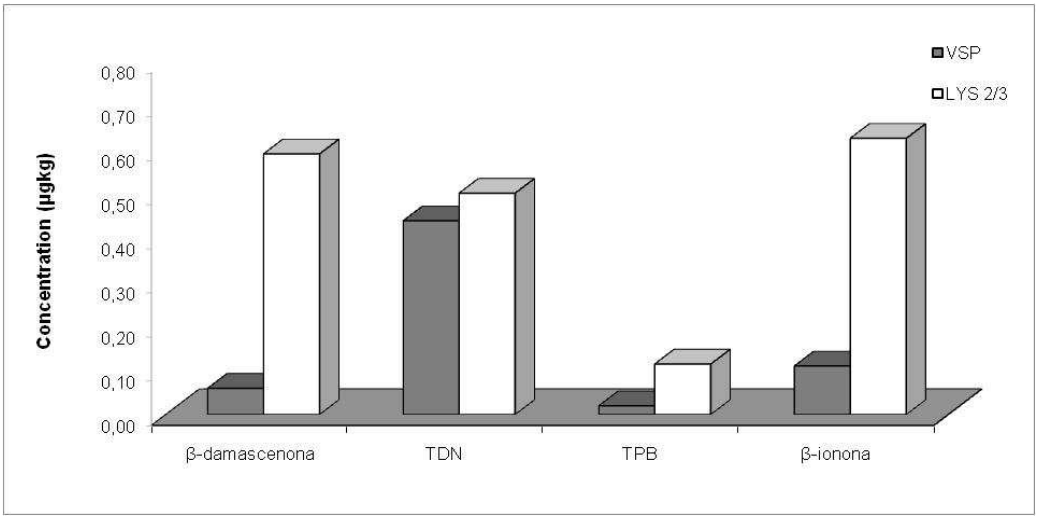

Fig. 4. Concentration in norisoprenoids in the glycosylated fraction of Touriga Nacional wines, in VSP and LYS 2/3 systems.

Conclusions

Touriga Nacional grapes and wines have higher contents in carotenoids, free and glycosylated fractions of terpenic and norisoprenoids compounds than Touriga Franca. VSP training system should be considered the most appropriate to obtain wines with high content in aroma compounds, once the free fraction of aroma was higher than the bounded fraction. However, if the objective is to obtain Port wines whose aromatic potential reveals during aging process, LYS 2/3 system should be chosen, due to the high levels of terpenic compounds and nor-isoprenoids existing in the bounded fraction.

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