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## INTRODUCTION

Terpenes and norisoprenoids are responsible for the varietal aroma of wines. Linalol, geraniol, nerol,  $\alpha$ -terpineol, citronellol and HO-trienol are the most odoriferous terpenic alcohols, which contribute to the floral aroma of wines. 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 forms; the terpene contents increase during alcoholic fermentation due to the  $\beta$ -glucosidase activity of yeasts. Norisoprenoids could be produced from direct carotenoid molecules degradation, such as  $\beta$ -carotene, lutein, neoxanthin and violaxanthin (1) and from the hydrolysis of glycoside molecules (2-4). Norisoprenoids compounds contribute to fruity, floral and spicy notes, and play a high sensory impact on wine aroma (5). Some norisoprenoids has been identified in wines, namely  $\beta$ -damascenone,  $\beta$ -ionone, vitispirane, 1,1,6-trimethyl-1,2-dihydroahtalene, 2,2,6-trimethylcyclohexanone and, more recently, (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (2,4,6-7).

## AIMS

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.

## MATERIALS 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 from *Quinta de Santo António* (The Fladgate Partnership Vinhos, S.A.). After harvest, the grapes were crushed, pressed and treated with sulphite solution to achieve 50 mg/L of SO<sub>2</sub>. 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<sup>®</sup> from Proenol, Lallemand), at 30 g/hL. All wines were produced using the same vinification procedure. Fermentations were carried out at 18 °C, 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.* (8). Free and glycosidic fractions of norisoprenoids and terpenes in grapes and Port wines were analysed by GC-MS (4).

## RESULTS

The grapes from TN variety presented higher content in carotenoids than Touriga Franca grapes (Table 1). For both training systems, the carotenoid content was similar. In TF grapes, VSP system allowed a high carotenoid content than LYS 2/3 system.

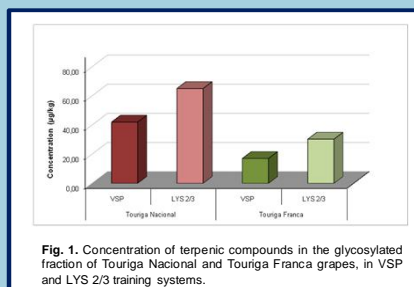
**Table 1.** Carotenoids content of Touriga Nacional and Touriga Franca grapes in VSP and LYS 2/3 training systems.

Compound ( $\mu$ g/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
$\beta$ -Carotene	737	739	658	500
Total carotenoids	1267	1259	903	717

nd – not detected

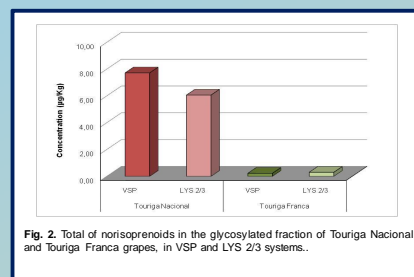
TN grapes presented a concentration in free terpene compounds higher in VSP system than in LYS 2/3. In TF grapes, the free fraction of terpenic compounds was only observed in LYS 2/3 system (data not shown), being the norisoprenoids only present in grapes in their glycosylated form.

In both vineyard training systems, the highest amounts of terpene compounds in the glycosylated fraction were obtained in TN grapes (Fig. 1). The VSP system produced grapes with lower amounts of these compounds than LYS 2/3 system.



**Fig. 1.** Concentration of terpenic compounds in the glycosylated fraction of Touriga Nacional and Touriga Franca grapes, in VSP and LYS 2/3 training systems.

As expected, TN showed higher contents of norisoprenoids in the glycosylated fraction than TF (Fig. 2). Despite the similar contents in norisoprenoids for both training systems, it was grapes from LYS 2/3 system that presented the highest amounts of  $\beta$ -damascenone and  $\beta$ -ionone, whereas the VSP system presented highest amounts of TDN and TPB.



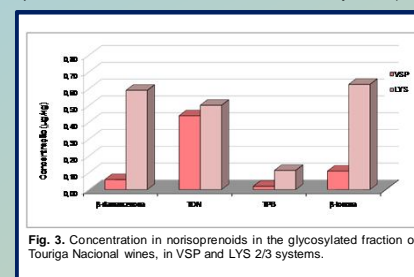
**Fig. 2.** Total of norisoprenoids in the glycosylated fraction of Touriga Nacional and Touriga Franca grapes, in VSP and LYS 2/3 systems.

TN wines showed a higher content of free terpenic compounds than TF wines (Table 2). TN 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 TF wines. As expected, glycosylated terpenic compounds in TF were lower than those obtained in TN, for both vineyards training systems.

**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 ( $\mu$ g/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

TN wines showed the highest content in free norisoprenoids in LYS 2/3 system (data not shown). As expected, TN wines showed higher content in glycosylated norisoprenoids than TF. Port wines obtained from LYS 2/3 system, showed higher composition in glycosylated norisoprenoids than those obtained from VSP system (Fig. 3).



**Fig. 3.** 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.

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, even if wines from VSP training system wines have little high contents in free fraction of aroma.

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