Determination of carotenoid profiles in grapes, musts and fortified wines from Douro varieties of *Vitis vinifera*. Role of alcoholic fermentation on carotenoid levels in fortified wines.

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Abstract

The most abundant carotenoids present in red grape varieties are β-carotene, lutein, violaxanthin, luteoxanthin and neoxanthin. These large non-aromatic molecules are known to be precursors of aroma-active substances responsible for the typical aroma of some grape varieties, their biological and chemical degradation leading to the formation of certain C13 nor-isoprenoid compounds such as α and β-ionone or β-damascenone - compounds associated with positive sensory attributes in wines. In order to study the profiles of carotenoids in grapes, musts and fortified wines from red Douro varieties of *Vitis vinifera* an HPLC method allows the determination of β-carotene, lutein and other xanthophylls (neoxanthin, violaxanthin and luteoxanthin). Despite the fact that the carotenoid levels found in fortified wines) were lower than those found in musts their presence in wines suggests the possibility of an *in-situ* conversion into nor-isoprenoids. In this work the carotenoid profile was followed during fermentation. Levels of lutein and β-carotene decreased drastically in the 2 first days - 510 μg/L to 125 μg/L for lutein and 980 μg/L to 215 μg/l for β-carotene. However neoxanthin, violaxanthin and luteoxanthin (which were undetectable in musts) increased during the 3 first days of fermentation. Subsequently levels decreased and carotenes were not detectable in wines fermented to dryness. As the port fermentation is, classically, interrupted by the addition of brandy when the sugar concentration reaches 100-150 g/L wines of this type can levels of neoxanthin, violaxanthin, luteoxanthin as high as 214 μg/L).

INTRODUCTION

- The two carotenoids which are most abundant in grapes are β-carotene and lutein, although violaxanthin and neoxanthin are also present in non-negligible amounts (Razungles *et al.*, 1993). These large non-aromatic molecules are known as precursors of aroma-active compounds of the nor-isoprenoid family including α and β-ionona and β-damascenone, responsable for the typical aroma of some grape varieties (Kotseridis, 1999).
- Several nor-isoprenoids have been identified in port: 2,2,6-trimethylcyclohexanone (Freitas *et al.*, 1999), and α and β-ionone (Silva Ferreira, 1993), and 1,1,6-trimethyl-1,2-dihydronaphthalene (Simpson, 1978).
- A number of mechanisms for the reaction and decomposition (via enzymatic processes, auto-oxidation and thermal decomposition) of carotenoids into nor-isoprenoids with 9 to 13 carbon atoms, have been described in foodstuffs. Certainly the best defined of these mechanisms are those which demonstrate production of nor-isoprenoids due to thermal treatments, in particular the ionone isomers, dihidroactinolide (DHA) and 1,1,6-trimethyl-1,2-dihydronaphthalene.
- The evolution of carotenoid contents of grapes during maturation has been studied by Razungles *et al.*, (1988). It has been shown that β-carotene and several xanthophylls (neochrome, neoxanthin, flavoxanthin and lutein), are abundant before veraison, although the amount of these compounds decreases during ripening (Razungles *et al.*, 1996).
- There is very little published data concerning the post-harvest (non-biochemical) degradation of carotenoids in grape-derived materials. This latter assertion together with initial work in our laboratory suggests that, in the case of ports, the persistence of carotenoids into the post fermentation phase, indicates a potential new source of aroma compounds in aged ports.

MATERIAL

1 - Grapes, musts and fortified wines used obtained from 5 cultivars (Touriga Nacional - TN, Touriga Francesa - TF, Tinta Roriz - TR, Tinto Cão – TC and Tinta Barroca - TB) harvested at two different sub regions (Cima Corgo - *CC* and Douro Superior - *DS*) of Douro Region. Grapes and musts were picked in October 1999 (harvesting date) and immediately frozen at –20ºC. Ports were available in February 2000.

2 - The evolution in carotenoids contents in musts was followed during fermentation performed by laboratory micro-vinification.

METHODS

Extraction of carotenoids (figure 1) and Chromatographic procedure (TLC and HPLC)

TLC - was performed in pre-coated silica gel 60 F254 aluminium sheets 20x20 cm (Merck, Germany) were used for the separation of carotenoids (Mendes-Pinto *et al.*, 2001) (figure 2)

HPLC - The quantification of carotenoids was done by HPLC, using an Beckman System Gold equipped with the 168 diode array detector. The wavelength select to cromatogram integration was 447 nm (figure 3) (Guedes de Pinho et al. 2001).

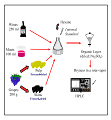
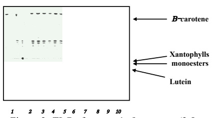


Fig.1.Carotenoid extraction methodology



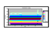


Figure 2. TLC of musts. 1: β-carotene (2.5 mg/ml); 2 : M5 (TNCC); 3 : M6 (TNDS); 4 : M7 (TBCC); 5 : M8(TBDS); 6 : M9 (TFCC); 7 : M10 (TFDS); 8 : M11 (TRCC); 9 : M12 (TRDS); 10 : M13 (TCCC) (on the lrft), scanning densitometry of musts (on the right).

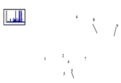
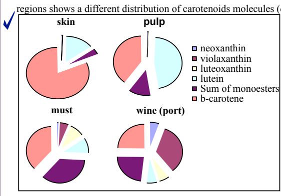


Figure 3. HPLC chromatogram of carotenoids of Tinta Roriz must. DO=447nm. 1. Neoxanthin, 2. Violaxanthin, 3. Luteoxanthin, 4. Unknown (lutein 5,6-epoxy ?) 5. Zeaxanthin, 6. Lutein, 7. Internal standard, 8. Xantophylls esters and 9. β-carotene.

RESULTS AND DISCUSSION

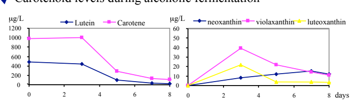
- The study of carotenoids in grapes shows very different profiles for skin and pulp. The skin contributes approximately 65% of total carotenoides (lutein, monoesters of xantophylls and β-carotene) while the contribution of pulp is only 35%. The analysis of musts coming from different varieties and from different sub-regions shows a different distribution of carotenoids molecules (data not shown).

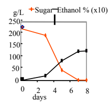


The carotenoid profiles of grapes, musts and wines are very different (figure 4). The abundance of xanthohylls in wines is higher than in musts and grapes. This fact is due to a differents solubility and extraction of these molecules. In ports after 3 days of fermentation brandy is added, the resultant wine having approximately 20% (v/v) alcohol, the stabilised , “unfinished” fermentation might explain the persistence of these compounds in the final wine.

Figure 4. Carotenoid profile during winemaking

- Carotenoid levels during alcoholic fermentation





During the alcoholic fermentation levels of β-carotene decreased whilst those of the xanthophylls (neoxanthin, violaxanthin and luteoxanthin) increased. The presence of these molecules in finshed ports might suggest a “pool” of aromatic-precursor compounds which could be converted into aroma-active substances during ageing. This scenario is currenly being tested in ports of the “vintage”category.

CONCLUSIONS

1. There are typical profiles of carotenoids in grapes, musts and wines. In grapes (skins and pulp) β-carotene and lutein are dominant. In musts xanthophylls esters and β-carotene exist in higher levels than lutein and other epoxy-xanthophylls.
2. In ports the quantitiy of carotenoids is lower than in grapes and musts, however lutein, neoxanthin and violaxanthin were found in appreciable amounts.
3. The data suggests a possible scenario of in which lutein and other xantophylls could directly be converted into nor-isoprenoids during aging of certain types of port.

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