Study of major aromatic compounds in port wines from carotenoid degradation

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Abstract

The carotenoids degradation and the formation of volatiles were examined by simulating Port wine aging. A two year old red Port wine was saturated with oxygen, supplemented with lutein and β-carotene and kept at 60 °C during 87 h. A similar study was performed in a model wine solution. Results showed that the percentage decrease in lutein levels was, respectively, 79% and 95%, in the wine model solution and in the Port wine, and 55% and 10% for β-carotene, indicating that lutein was more sensitive to degradation than β-carotene. Two other unknown degradation carotenoid compounds were identified by HPLC/DAD (reverse phase λ_{max}: 422; 445; 475 and 422; 445; 472) in the lutein supplemented wine. Levels of β-ionone and β-cyclocitral increased (2.5 times) in both, wine and wine model solution, supplemented with β-carotene. Along with these compounds, the same behaviour was observed in β-damascenone in the supplemented lutein wine and wine model solution. New insights were provided into the understanding of aroma modifications occurring during Port wine aging. The relationship between carotenoid molecules (β-carotene and lutein) and some volatiles has also been provided.

Introduction

The presence of carotenoids in grapes is well established. It has been demonstrated that levels of β-carotene and several xanthophylls (neoxanthin, flavoxanthin and lutein) are abundant before veraison, falling dramatically after this period (Razungles, Babic, Sapis, & Bayonove, 1996; Razungles, Bayonove, Cordonnier, & Baumes, 1987; Razungles, Bayonove, Cordonnier, & Sapis, 1988). Carotenoids are known as precursors of norisoprenoids, responsible for the typical aroma of same grape varieties. It was reported that norisoprenoids could originate from direct degradation of carotenoid molecules such as β-carotene, lutein, neoxanthin and violaxanthin (Kanasawud & Crouzet, 1990; Kotseridis, Baumes, & Skouroumounis, 1998; Marais, van Wyk, & Rapp, 1989; Mordi et al., 1991; Winterhalter, 1992). Recently it was demonstrated that β-damascenone can be formed directly from 9′-*cis*-neoxanthin by chemical oxidation using high temperatures (Bezman et al., 2005). These norisoprenoids can also be liberated from the hydrolysis of glycoside molecules (Di Stefano et al., 1998; Skouroumounis, Massy-Westropp, Sefton, & Williams, 1992). β-damascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and vitispirane have been identified in wine some years ago (Simpson, 1978; Singleton, Trousdale, & Zaya, 1979).

More recently, 2,2,6-trimethylcyclohexenone, another non-megastigmane norisoprenoid, was reported in wines (Freitas, Ramalho, Azevedo, & Macedo, 1999). All these molecules have an important sensorial impact on wine aroma as they have very low olfactory perception thresholds (Etievant, 1991).

As carotenoid compounds have been found in Port wines (Guedes de Pinho, Ferreira, Mendes Pinto, Gomez Benitez, & Hogg, 2001), and knowing that these wines can have an extended time of storage, it can be presumed

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that these molecules could be precursors of volatiles. The aim of this study was to determine carotenoids degradation and the formation of volatiles by simulating Port wine aging. This work could provide some new insights into the study of major aromatic compounds in Port wines from carotenoid degradation.

Material and methods

Wine material and treatments

A volume of 6000 mL of two year old red Port wine (pH 3.4) was saturated with oxygen (6.5 mg/L) and divided into three equal portions (2000 mL each): (i) a first portion corresponded to the untreated wine (control), (ii) a second portion was supplemented with lutein, and (iii) a third portion was supplemented with β-carotene. Each set was kept for 87 h at a storage temperature of 60 °C. A similar protocol was made in a model wine solution (pH 3.4 with tartaric acid and 20% ethanol).

Extraction and determination of carotenoids

Wine material. Two hundred and fifty millilitres of wine were spiked with 100 μL of internal standard, 170 mg/L of β-apo-8′-carotenal (FLUKA, Lisbon, Portugal) (10810). Extraction was carried out with 40 mL of ether/hexane (1:1, v/v), "HPLC Grade" (MERCK, Lisbon, Portugal), agitated for 30 min. The extraction was repeated two more times with 20 mL of ether/hexane (30 min each). The final combined extract was concentrated to dryness (rotavapor) and resuspended in 0.5 mL of acetone/hexane (1:1, v/v) for HPLC determination. Light exposure was minimized during sample preparations in order to avoid photoisomerisation (Oliveira, Barbosa, Silva Ferreira, Guerra, & Guedes de Pinho, 2006; Oliveira, Silva Ferreira, Costa, Guerra, & Guedes de Pinho, 2004; Oliveira et al., 2003).

HPLC. A Beckman Model 126 quaternary solvent system, equipped with a System 32 Karat software and a 168 rapid-scanning (Mem Martins Lisbon, Portugal), UV–vis photodiode array detector (Mem Martins, Lisbon, Portugal), was used. The absorption spectra were recorded between 270 and 550 nm. Stationary-phase: Nova-Pack C18 60 Å 4 μm particles (3.9 ∗ 300 mm), WATERS (Sacavém, Portugal). Mobile-phase: solvent A, ethyl acetate (MERCK pure grade); solvent B, acetonitrile/water (9:1 v/v) (MERCK pure grade and pure water), flow rate = 1 mL/min. The following gradient was employed: 0–31 min (0–60% A); 31–46 min (60% A); 46–51 min (60–100% A); 51–55 min (100% A); 55–60 min (100–0% A); 60-65 min (0% A).

Solid phase microextraction (SPME) for volatile compound analysis

The fibre employed was coated with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 50/30 μm (Supelco, Bellefonte, PA, USA). For each SPME analysis, 20 mL of sample was placed in a vial of 40 mL capacity with a small stir magnet, at 1300 rpm, spiked with an internal standard (20 μL of methanolic solution of 3-octanol, 47.7 mg/L) and immersed in a water-bath at 35 °C. The SPME needle then pierced the septum and the fibre was extended through the needle to place the stationary phase in contact with the headspace of the sample during 90 min. Finally, it was removed from the vial and inserted into the injection port of the gas chromatograph for 10 min. The extracted chemicals were thermally desorbed, at 220 °C, and transferred directly to the analytical column. Fibres were cleaned before each microextraction process to prevent contamination by inserting the fibre in the auxiliary injection port at 220 °C for 30 min (Silva Ferreira & Guedes de Pinho, 2003).

Gas Chromatography analysis-mass spectrometry

Samples were analysed using a Varian CP-3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a Varian Saturn 2000 mass selective detector and a Saturn GC/MS workstation software version 5.51. The column used was a STABILWAX-DA (60 m × 0.25 mm, 0.25 μm) fused silica (Restek, Bellefonte, PA, USA). The injector port was heated to 220 °C. The split vent was opened after 30 s. The oven temperature was 40 °C (for 1 min), then increased at 2 °C/min–220 °C and held for 30 min. The carrier gas was Helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow. All mass spectra were acquired in the electron impact (EI) mode with the Ion Trap detector set as follows: transfer line, manifold and trap temperatures 230 °C, 45 °C and 170 °C, respectively. The mass range was 33 m/z to 350 m/z, with a scan rate of 6 scan/s, and without solvent delay. The emission current was 50 μA, and the electron multiplier was set to the auto-tune procedure. The maximum ionization time was 25,000 μs with an ionization storage level of 35 m/z. The analysis was performed in Full Scan mode (Silva Ferreira, 1998). Quantitative analysis was preformed by selected ion current mode. Ions selected were respectively for 3-octanol, $m/z = 83$; β-ionone, $m/z = 177$; β-damascenone, $m/z = 121$; β-cyclocitral, $m/z = 137$.

Identication of molecules

Carotenoids

Carotenoids were identified by comparison with commercially available standards, β-carotene (Sigma 95%, synthetic) (C-9750), lutein (Sigma 70%, from alfalfa) (X-6250) and UV–vis photodiode array spectra.

Aromatic molecules

Aromatic molecules were identified by comparison with mass spectra obtained from the sample with those from pure commercially available standards injected using the same conditions and by comparing the Kovats indices and the mass spectra present in the NIST 98 MS library.

Pure standards were purchased from Sigma–Aldrich (Lisbon, Portugal): β-ionone (I-1, 260-3) (96%) and β-cyclocitral (432-25-7) (>90%). The β-damascenone was kindly supplied by Firmenich (Geneva, Switzerland).

Determination of dissolved O2

Dissolved O_2 was measured directly using an "YSI Oxygen Probe – 5010-W", coupled to a 5000 dissolved oxygen instrument. This model is designed to fit directly in the bottleneck for direct measurement of wine bottles.

Results and discussion

Carotenoid degradation

Fig. 1 illustrates the decrease of lutein (peak a) and the increase of two other unknown degradation carotenoid compounds (peak c and d) identified by HPLC/DAD (reverse phase λ_{max}: 422; 445; 475 and 422; 445; 472), during the experimental period, in the lutein supplemented wine. These unknown compounds could be originated from lutein degradation and their characterization by UV–vis spectra is present in Figs. 2 and 3.

Results show that the percentage of decrease in lutein levels was, respectively, 79% and 95% (Fig. 1 and Table 1) in the wine model solution (control) and in the Port wine. Fig. 4 shows the decrease of β-carotene (peak e) during the experimental period, in the β-carotene supplemented wine. Results have shown that the percentage of decrease on β-carotene levels was, respectively, 55% and 10% (Fig. 4 and Table 1) in the wine model solution (control) and in the Port wine. These results seem to indicate that lutein is more sensitive to degradation than β-carotene.

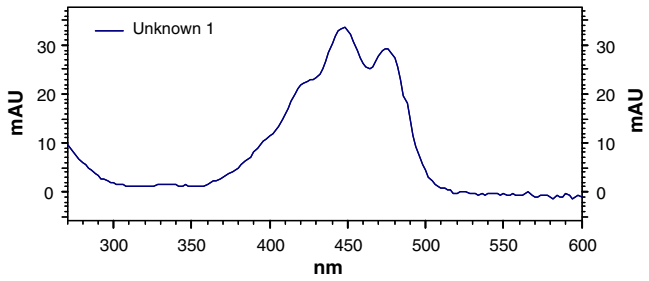

Fig. 2. Identification of unknown 1 (λ_{max}: 422; 445; 475).

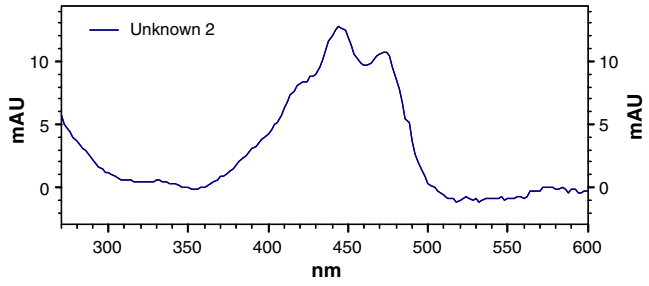

Fig. 3. Identification of unknown 2 (λ_{max}: 422; 445; 472).

Volatiles formation

Levels of β-ionone and β-cyclocitral increased (2.5 times) in both wine (Figs. 5 and 6) and wine model solution supplemented with β-carotene. Along with these compounds, the same behaviour was observed on β-damascenone in the supplemented lutein wine and wine model solution (Fig. 7). β-ionone can participate in wine aroma with a very low perception threshold of 90 ng/L (in a model base wine) Kotseridis (1999). Its descriptor is "violet" and it can be formed by β-carotene degradation (Kanasawud &

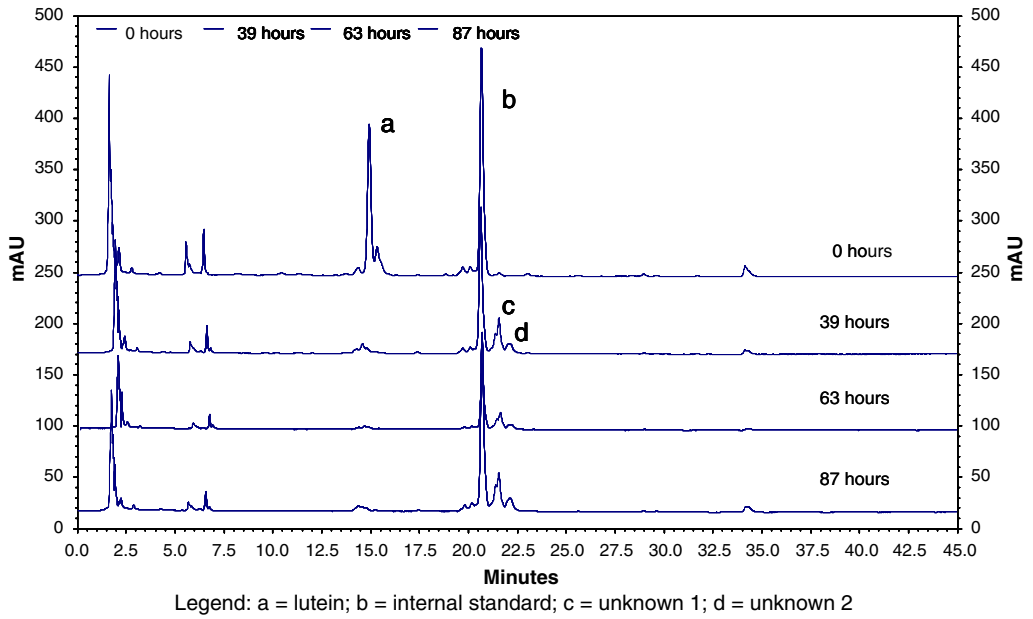

Fig. 1. Lutein degradation in Port wine during 87 h at 60 °C. a = lutein; b = internal standard; c = unknown 1; d = unknown 2.

Table 1
Carotenoid degradation in the wine model solution (control) and in the Port wine

	Time of storage	Lutein (μg/L)	Unknown 1 (∗)	Unknown 2 (∗)	β-Carotene (μg/L)
C	0	nd	nd	nd	nd
	39	nd	nd	nd	nd
	63	nd	nd	nd	nd
	87	nd	nd	nd	nd
C + L	0	337.0	nd	nd	nd
	39	135.2	48.8	17.2	nd
	63	36.6	34.4	14.4	nd
	87	69.3	36.0	16.6	nd
C + C	0	nd	nd	nd	284.0
	39	nd	nd	nd	151.3
	63	nd	nd	nd	92.7
	87	nd	nd	nd	128.5
WC	0	nd	nd	nd	nd
	39	nd	nd	nd	nd
	63	nd	nd	nd	nd
	87	nd	nd	nd	nd
W + L	nd	337.0	nd	nd	nd
	39	30.0	173.6	47.4	nd
	63	19.3	191.5	54.2	nd
	87	16.0	129.1	40.6	nd
W + C	0	nd	nd	nd	284.0
	39	nd	nd	nd	205.9
	63	nd	nd	nd	239.5
	87	nd	nd	nd	259.3

(∗) – Concentration expressed in μg/L of lutein.C = control; C + L = control + lutein; C + C = control + β-carotene.WC = wine control; W + L = wine + lutein; W + C = wine + β-carotene. nd – Not detected. The extraction and examination of carotenoids were made in duplicate and the results are given in the form of mean.

Crouzet, 1990) or by its sugar precursor hydrolysis (Kotseridis, 1999). For this reason, and the fact that it occurred

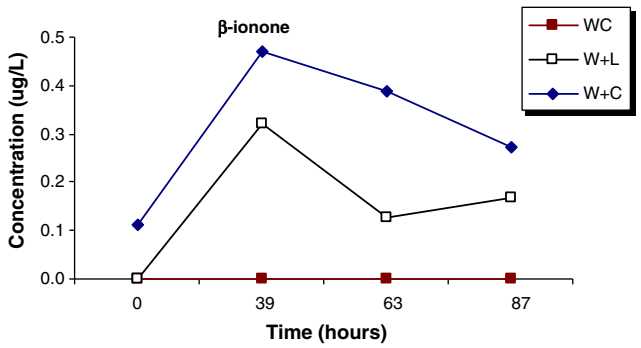

Fig. 5. β-Ionone formation in Port wine during 87 h of storage. WC = wine control; W + L = wine + lutein; W + C = wine + β-carotene.

well above its threshold value, β-ionone may be considered as a contributor for Port wine aroma. Along with β-ionone, β-damascenone is recognised, as well, as having a very low aroma perception threshold and could be considered as a supplier for wine aroma.

These data suggest that both lutein and β-carotene can contribute to the aroma of Port wines, as they can degrade into small molecules like norisoprenoid compounds. It was reported that norisoprenoids could come from the direct degradation of carotenoid molecules such as β-carotene and lutein, (Kanasawud & Crouzet, 1990; Kotseridis et al., 1998; Marais et al., 1989; Mordi et al., 1991; Winterhalter, 1992). In this work, β-ionone and β-cyclocitral where identified as the major released aroma molecules in a Port wine supplemented with β-carotene with increases up to 0.2 and 0.5 μg/L, respectively, for β-ionone and β-cyclocitral. These results are in agreement with previous studies in other matrixes like fungi (Zorn, Langhoff, Scheibner, & Berger, 2003). Furthermore, β-damascenone has been firstly identified in Port wine supplemented with lutein. All these molecules have an important sensorial

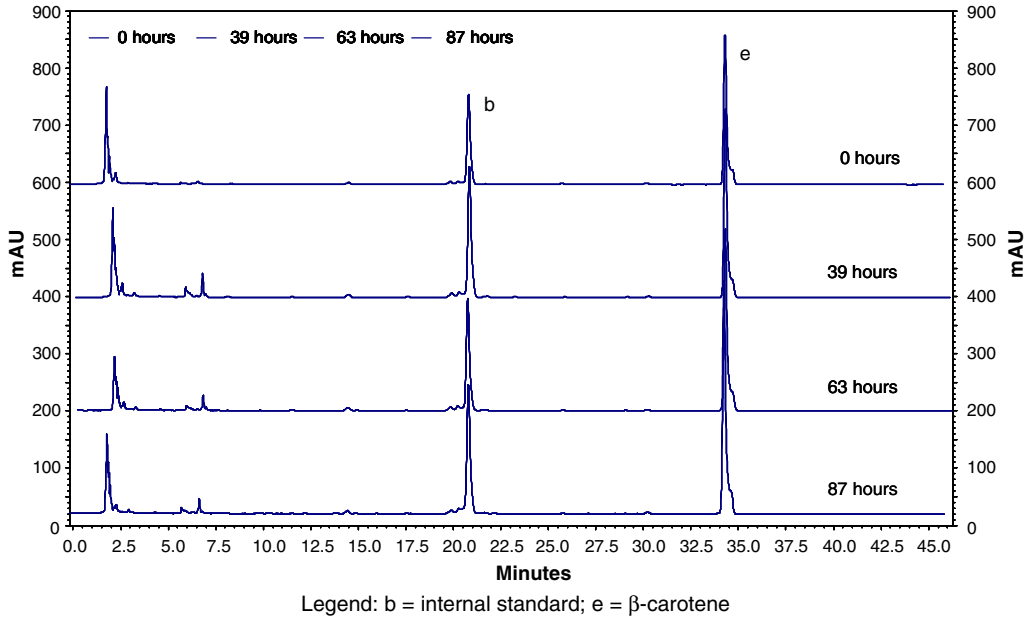

Fig. 4. β-Carotene degradation in Port wine during 87 h at 60 °C. b = internal standard; e = β-carotene.

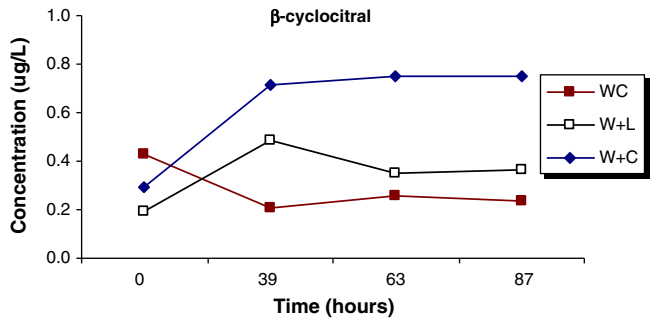

Fig. 6. β-Cyclocitral formation in Port wine during 87 h of storage. WC = wine control; W + L = wine + lutein; W + C = wine + β-carotene.

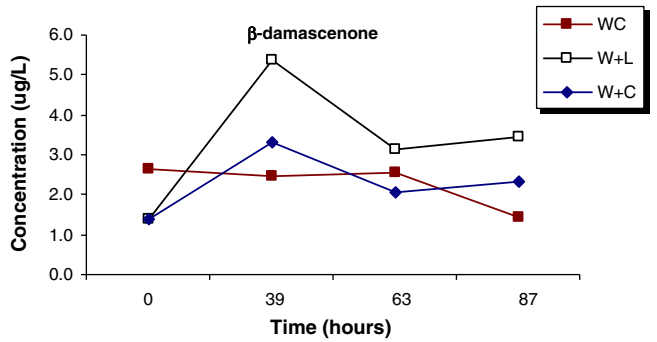

Fig. 7. β-Damascenone formation in Port wine during 87 h of storage. WC = wine control; W + L = wine + lutein; W + C = wine + β-carotene.

impact on wine aroma as they have very low olfactory perception thresholds. Further research should be done in order to assess the possible relation between carotenoids evolution and the direct conversion into norisoprenoid compounds during Port wine ageing.

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