Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 99 (2006) 51–56
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of β-damascenone in alcoholic beverages by reversed-phase liquid chromatography with ultraviolet detection

J.R. Carneiro, J.A. Ferreira, L.F. Guido, P.J. Almeida, J.A. Rodrigues, A.A. Barros *

Requimte, Departamento de Química, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal

Received 22 November 2004; accepted 13 July 2005

Abstract

A new method of analysis was previously developed by this group for the determination of β-damascenone in beer samples. The method consisted of a beer steam distillation, followed by an extraction/concentration step using a C_{18} SPE column and, finally, analysis by reversed-phase liquid chromatography with detection of β-damascenone at its 227 nm UV-absorption maximum. This work describes further developments of the previous methodology and its application to the identification and quantification of β-damascenone in some other beverages, such as wine, whiskey and brandy. β-Damascenone concentration was monitored during a forced ageing assay of several wines from different regions of Portugal and the influence of heat treatment of wine on β-damascenone concentration was studied. The effect of pH on β-damascenone concentration is also discussed.

Keywords: β-damascenone; Wine; Beer; Whisky; Brandy; Food analysis

1. Introduction

β-Damascenone (8E-megastigma-3,5,8-trien-7-one), a C_{13}-norisoprenoid belonging to the family of the rose ketones, was first isolated from Bulgarian rose oil (Demole, Enggist, Säuberli, Stoll, & Kovats, 1970) and later found in the essential oils of many other natural products. β-Damascenone is a highly odoriferous compound with a powerful and pleasant fragrance, being an important compound in the perfume and flavouring industries. As it is characterised by having a very low sensory threshold in water (approximately 0.02–0.09 $\mu g l^{-1}$) (Buttery, Teranishi, Ling, & Turnbaugh, 1990), β-damascenone is considered to be a key odour compound in many alcoholic beverages (whisky, brandy, rum, wine and beer), imparting a "stewed apple", "fruity-flowery" and honey-like character. β-Damascenone has also been identified in many fruits (apples, peaches, grapes, passion fruits and raspberries) and some non-alcoholic beverages, such as tea and coffee.

An increase in β-damascenone concentration by heat treatment has been reported for tea (Kumazawa & Masuda, 2001), wine (Kotseridis, Baumes, & Skouroumounis, 1999) and, recently, beer (Chevance, Guyot-Declerck, Dupont, & Collin, 2002; Guido et al., 2004). Kotseridis et al. (1999) observed that free-damascenone levels were almost doubled by heat treatment of Merlot wines and Guido et al. (2004) showed that, in Portuguese beers, β-damascenone could be used as a good analytical marker of beer ageing induced by heat. Although the mechanisms responsible for the formation/liberation of β-damascenone in nature have not yet been completely elucidated, the hydrolytic breakdown of complex secondary metabolites derived from carotenoids, such as neoxantin, is the most likely

* Corresponding author. Tel.: +351 2260 829 39; fax: +351 2260 829 59.
E-mail address: ajbarros@fc.up.pt (A.A. Barros).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.07.024

hypothesis (Isoe, Katsumura, & Sakan, 1973; Puglisi, Elsey, Prager, Skouroumounis, & Sefton, 2001; Skouroumounis & Sefton, 2000). In beer, the formation/liberation of β-damascenone is enhanced by the adjustment of pH to more acidic values (Gijs, Chevance, Jerkovic, & Collin, 2002; Guido et al., 2004), thus supporting the hypothesis that an acid hydrolysis is responsible for the development of β-damascenone.

Although GC–MS is the most widely used methodology for the determination of β-damascenone in beverages (Chevance et al., 2002; Kotseridis et al., 1999), LC–UV methods can also be used (Guido et al., 2004). As β-damascenone levels found in beverages are usually very low, a pre-concentration step of this compound is required before chromatographic analysis. In some cases, these sample treatments are expensive, very laborious and time-consuming. In recent reports (Guido et al., 2004; Santos et al., 2003), we described a new methodology for the quantification of β-damascenone in beer samples, involving a steam distillation for extraction of β-damascenone from beer, followed by a solid phase extraction concentration step and, finally, analysis by high performance liquid chromatography with UV detection. In this work, further developments of the previous methodology and its application to the identification and quantification of β-damascenone in some other beverages (wine, whisky and brandy) are reported. A more specific study was performed to access the influence of heat treatment and of the pH medium on the concentration of β-damascenone.

2. Materials and methods

2.1. Instrumentation

2.1.1. Extraction

Solid phase extraction (SPE) columns of 200 mg Chromabond C_{18} were obtained from Macherey–Nagel. The steam distillation system used was made with ordinary laboratory glass material. A PTFE tube was used to connect the steam generator vessel to the sample vessel.

2.1.2. HPLC analysis

A Gilson HPLC instrument was used, consisting of a 307 pump, a 115 UV variable wavelength detector, selected at 227 nm, a Rheodyne 7125 injection valve with a 20-μl and a Spectra Physics Data Jet CH1 integrator. A precolumn ChromSep Guard ODS (10 mm × 4 mm, 5 μm particle size) and a column Nucleosil ODS (250 mm × 4.6 mm, 5 μm particle size) from Varian were used. A water–acetonitrile (45:55, v/v) mixture was used as mobile phase at a flow-rate of 1.00 ml.min^{-1}. The mobile phase was filtered and degassed in a vacuum filter holder, Schleicher & Schuell GV 050/0, with 0.2 mm Schleicher & Schuell NL 16 membrane filters.

2.2. Reagents

Acetonitrile (HPLC grade), ethanol (analytical reagent grade), hydrochloric acid (37%), sodium hydroxide (analytical reagent grade) and sodium chloride (analytical reagent grade) were purchased from Merck. β-Damascenone (>95%) was kindly provided by SGP Selin, Grasse, France. The water used to prepare all solutions was deionised, distilled and further purified in a Millipore system (Simplicity 185). Stock solutions of β-damascenone were prepared weekly in water–acetonitrile (50:50, v/v). In this medium, β-damascenone is soluble and stable. An antifoaming agent, based on silicon from Merck, was used to prevent the formation of foam during beer distillation.

2.3. Samples

Wine, whisky and brandy samples were purchased from local supermarkets. Wine samples from different regions of Portugal (Table 1) were subjected to artificial ageing (37 °C in the dark for 1, 2 and 4 weeks) and β-damascenone content was measured for fresh and artificially aged samples. Beer samples were kindly provided by the local brewery, UNICER—Bebidas de Portugal SGPS S.A.

2.4. Experimental procedure

β-Damascenone was determined in beer, wine, whisky and brandy by the method previously developed by this group (Guido et al., 2004; Santos et al., 2003). The method is divided into three main steps: (1) steam distillation of the beverage sample; (2) passage of the collected distillate through a SPE C_{18} column; (3) elution and analysis by HPLC with UV detection. Before distillation, a proper aliquot of wine, whisky and brandy was diluted with ultra-purified water to 250 ml, in contrast to beer (250 ml) that was distilled without any dilution. The ionic strength of all samples was adjusted by the addition of 2.9 g of sodium chloride. For beer samples,

Table 1
Portuguese wines in which β-damascenone was quantified

Wine	Name	Region	Year
White	Wine I	Alentejo	2003
	Wine II	Alentejo	2003
	Wine III	Douro	2001
	Wine IV	Douro	2003
Red	Wine I	Alentejo	2003
	Wine II	Alentejo	2003
	Wine III	Douro	2001
	Wine IV	Douro	2001
Porto	Wine I	Douro	2002
	Wine II	Douro	2002

one drop of an antifoaming agent was added to prevent the formation of foam. The collected distillate (15 ml) was diluted to 100 ml with ultra-purified water and was passed through the SPE column. After draining the column by vacuum, the retained compounds were extracted with 1 ml of acetonitrile and 20 μl of this extract were injected into the HPLC column. Fig. 1 illustrates a typical chromatogram obtained in the analysis of a wine extract.

3. Results and discussion

3.1. Efficiency of the distillation process for the determination of β-damascenone in alcoholic beverages

The different ethanolic contents (between 5% and 40%) of the alcoholic beverages analysed during this work (wine, beer, whisky and brandy) can influence the efficiency of the distillation process. A specific study was performed to evaluate the influence of the ethanolic content on the recovery of β-damascenone during the distillation process. In this study, different volumes of ethanol were added to several aliquots of a white wine sample (125 ml), the solution was diluted to 250 ml with ultra-purified water and the concentration of β-damascenone in each sample was determined by the proposed methodology. As can be seen in Fig. 2, the recovery of β-damascenone is highly dependent on the ethanolic content of the distilled samples, with lower recoveries being obtained for the samples with higher ethanolic contents. Indeed, the recovery of β-damascenone is above 80% in the sample with 6% of ethanol and does not exceed 10% in the sample with 40% of ethanol. Similar studies were performed for a red wine sample and for a whisky sample and identical results were obtained (data not shown). In view of the obtained results, a proper dilution of wine, whisky and brandy must be done before distillation, so that the ethanolic content in the sample submitted to distillation does not exceed 6%.

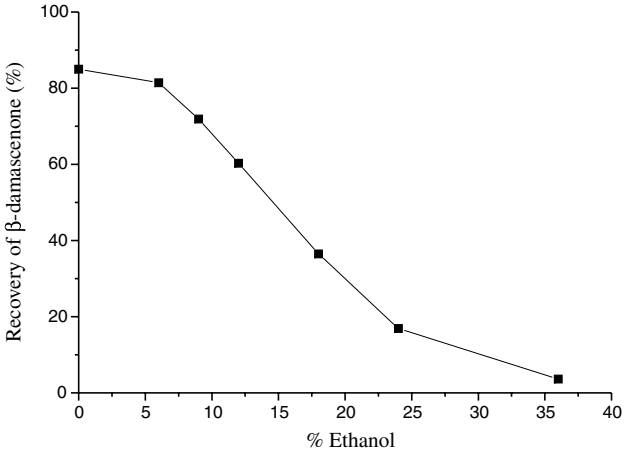

Fig. 2. Influence of ethanol content on the recovery, by steam distillation, of β-damascenone from a white wine sample (for 0 % of ethanol, an a model solution of β-damascenone was analysed).

It is well-known that high temperatures can induce the formation of β-damascenone from its precursors. For that reason, the possibility of an extra generation of β-damascenone during the steam distillation process

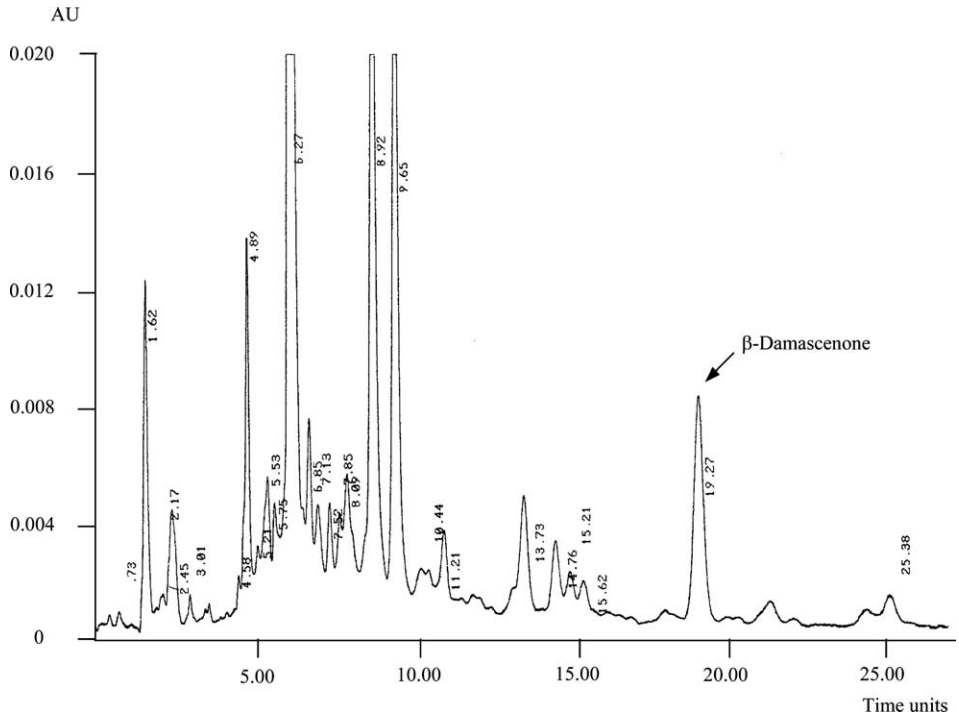

Fig. 1. Typical chromatogram of a wine extract (white wine IV).

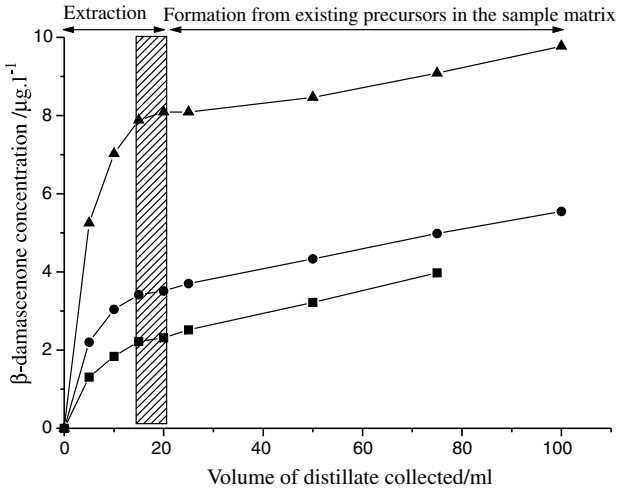

Fig. 3. Peak area of β-damascenone as function of the volume of distillate collected during steam distillation of wine (■) and wine spiked with 4.0×10^{-9} moll^{-1} (●) and with 1.6×10^{-8} moll^{-1} (▲) of β-damascenone.

was investigated. Aliquots of a 1:1 diluted wine sample (250 ml) were analysed, collecting different volumes of distillate and determining the β-damascenone concentration in each collected distillate-fraction. This study was repeated for two samples spiked with two different concentrations of β-damascenone. As Fig. 3 shows, using 250 ml test-samples and steam distillation, all the existing β-damascenone is extracted in the first 15–20 ml of distillate. Moreover, for larger volumes of distillate there is a smooth increase of the signal of β-damascenone, indicating that this compound is being slowly formed. This study was also performed for beer samples and similar results were obtained (data not shown). So, if a 250 ml beverage sample (diluted or not) is analysed, 15 ml seems to be the convenient volume of distillate to be collected, in order to avoid the formation of extra β-damascenone from its precursors.

3.2. Determination of β-damascenone in some alcoholic beverages

The methodology used for the determination of β-damascenone in wine, in whisky and in brandy was initially developed for the determination of this compound in beer (Guido et al., 2004). As an ethanol content above 6% affects the efficiency of the distillation process, a proper dilution of wine, whisky and brandy must be done before distillation to avoid this problem.

For an accurate quantification of β-damascenone, it is necessary to take into consideration any losses of this compound that can occur during the extraction/concentration stage of analysis. In order to test this, 10 samples of each beverage (red wine I, white wine II, whisky and brandy) were fortified with various aliquots of β-damascenone stock solution and subjected to analysis. High recoveries of β-damascenone were observed for all the samples, ranging between $81 \pm 4\%$ for brandy and $92 \pm 3\%$ for red wine I. Anyway, β-damascenone was quantified in wine, whiskey and brandy by the standard additions method (5 additions), with the standard being added to the beverage before distillation. As can be seen in Table 2, the slopes obtained for the different standard additions curves were very similar (ranging from 1.26×10^{12} to 1.50×10^{12}), showing that there are no significant interferences from the sample matrix. β-Damascenone concentration, with a 95% confidence limit, was determined according to Miller and Miller (1998). The highest level of this compound was found in whisky (16.3 ± 1.9 μgl^{-1}), followed by brandy (8.1 ± 1.4 μgl^{-1}) and finally the different Portuguese wines. β-Damascenone was also identified and quantified in the remaining wines listed in Table 1. In these wines, β-damascenone levels ranged from 1.3 ± 0.2 μgl^{-1} to 4.5 ± 0.9 μgl^{-1} for white wines, from 0.50 ± 0.08 μgl^{-1} to 2.8 ± 0.5 μgl^{-1} for red wines and from 3.3 ± 0.8 μgl^{-1} to 3.9 ± 0.8 μgl^{-1} for Porto wines. No correlation was found between the concentration of β-damascenone and the type/region of the analysed wine.

3.3. Effect of heat treatment of wine on the concentration of β-damascenone

β-Damascenone was recently described as a good analytical marker of beer ageing induced by heat (Guido et al., 2004). Storing beers at high temperatures can lead to the formation/liberation of β-damascenone, resulting in an increase of the concentration of this compound. An increase of β-damascenone levels was also reported for heat-treated wines by Kotseridis et al. (1999). These authors observed that β-damascenone levels were almost doubled by heat treatment in a French wine.

In order to assess the influence of heat treatment on the concentration of β-damascenone found in wine, eight Portuguese wines were stored at 37 °C for 1 week, 2 weeks and 4 weeks and the level of β-damascenone was monitored throughout the storage time (all analyses were performed in triplicate). The high storage temperature of wine did not cause a significant increase in β-damascenone concentration (Fig. 4), in contrast to what happens with beer. Indeed, the level of β-damascenone remained more or less constant throughout the storage time in almost all of the studied wines. An increase in β-damascenone concentration was only observed for red wine III (from 0.50 ± 0.08 μgl^{-1} to 0.92 ± 0.09 μgl^{-1} after 4 weeks) and for red wine IV (from 0.57 ± 0.08 μgl^{-1} to 1.1 ± 0.1 μgl^{-1} after 4 weeks). It is also noteworthy that, in white wine I, the β-damascenone concentration decreased during the 4-week storage (from to 1.8 ± 0.2 μgl^{-1} to 0.92 ± 0.09 μgl^{-1}).

A more drastic study was performed to assess the influence of heat treatment on β-damascenone concen-

Table 2
Parameters of the standard additions curves, final concentrations of β-damascenone in the beverage samples and repeatability of the method

Sample	Curve equation ($y = a + bx$)		r	[β-damascenone][a] ($\mu g l^{-1}$)	Method CV[b] (%)
	a	$10^{-12}b$			
Red wine I[c]	9504	1.31	0.9989	2.8 ± 0.5	3.9
White wine II[c]	14970	1.50	0.9985	3.8 ± 0.6	5.7
Porto wine I[c]	11107	1.28	0.9952	3.3 ± 0.8	8.7
Whisky[d]	20465	1.49	0.9993	16.3 ± 1.9	4.2
Brandy[d]	8589	1.26	0.9996	8.1 ± 1.4	6.2

[a] 95% confidence limits, determined according to Miller and Miller (1998).
[b] Coefficient of variation of the method (10 replicates).
[c] 125 ml of wine diluted to 250 ml with ultra-purified water.
[d] 40 ml of whisky/brandy diluted to 250 ml with ultra-purified water.

tration. Wine samples (white wine II and red wine I) were heated under reflux during long periods of time (1, 2 and 3 h) and the concentration of β-damascenone was determined in triplicate for each sample. A similar study was performed for beer samples. As can be seen in Fig. 5, the continuous production of β-damascenone by the sample matrix was higher for beer, where β-damascenone concentration increased nearly thrice (from $2.8 \pm 0.5\ \mu g l^{-1}$ to $7.5 \pm 0.9\ \mu g l^{-1}$) after heating under reflux during 3 h. Smaller increases were observed in β-damascenone concentration after the same 3 h under reflux in the wine samples: from $3.6 \pm 0.4\ \mu g l^{-1}$ to $7.0 \pm 1.2\ \mu g l^{-1}$ in white wine II and from $2.2 \pm 0.3\ \mu g l^{-1}$ to $3.2 \pm 0.4\ \mu g l^{-1}$ in red wine I. Based on these results, it seems that beer has more precursors capable of originating β-damascenone by heat treatment than wine.

Although the mechanisms responsible for the appearance of β-damascenone in beer and in wine have not yet been completely elucidated, it is generally believed that an acid hydrolysis is responsible for the formation/liberation of this compound (Isoe et al., 1973; Puglisi et al., 2001; Skouroumounis & Sefton, 2000). The effect of wine pH in the concentration of β-damascenone was evaluated for white wine II and for red wine I. Several

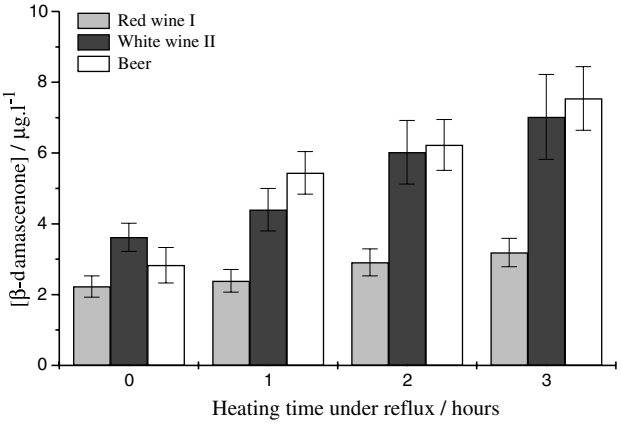

Fig. 5. Effect of heating time (under reflux) on the production of β-damascenone in red wine I, in white wine II and in beer.

aliquots of these two wines were analysed after pH adjustment. As can be seen in Fig. 6, the concentration of β-damascenone found in red wine I was almost doubled by adjusting the pH to 2 ($2.5 \pm 0.4\ \mu g l^{-1}$ at wine pH against $4.8 \pm 0.7\ \mu g l^{-1}$ at pH 2). In white wine II there was a similar increase when pH was adjusted to 2 ($3.6 \pm 0.4\ \mu g l^{-1}$ at white wine II pH against

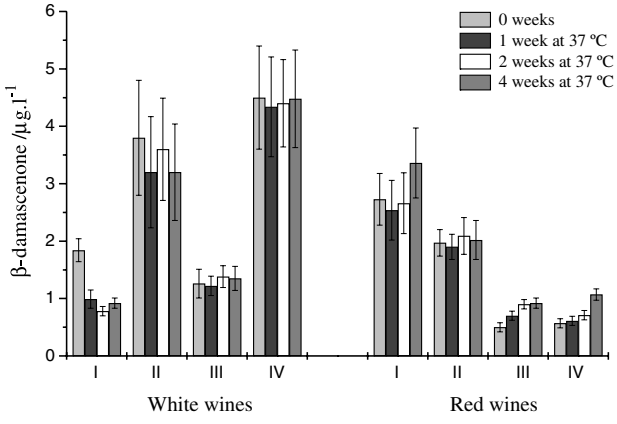

Fig. 4. Evolution of β-damascenone concentration during wine storage at 37 °C.

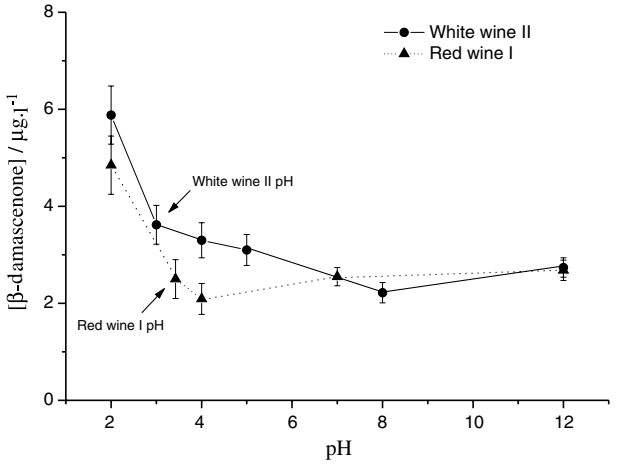

Fig. 6. Effect of pH on the concentration of β-damascenone found in white wine II and in red wine I.

$5.9 \pm 0.7\ \mu g l^{-1}$ at pH 2). A similar study, performed by Guido et al. (2004) for beer samples, has shown that, in beer, a 5-fold increase occurred when pH was adjusted to 2 ($3.2 \pm 0.4\ \mu g l^{-1}$ at beer pH against $17.3 \pm 2.0\ \mu g l^{-1}$ at pH 2). These results are in agreement with the hypothesis that an acid hydrolysis can lead to the development of β-damascenone in beer and in wine. Again, these results seem to indicate that wine has fewer precursors capable of originating β-damascenone than beer.

4. Conclusions

A method, previously developed by this group for the determination of β-damascenone in beer, was here applied for the determination of this compound in wine, in whisky and in brandy. In contrast to what happens in beer, these beverages must be diluted before distillation in order to achieve high recoveries of β-damascenone during the extraction step. During the pre-treatment of the sample, recovery studies, performed in wine, in brandy and in whisky samples, have shown that a recovery above 80% is obtained for β-damascenone. No significant interferences from the sample matrix were observed in the determination of β-damascenone.

An extended storage of wine samples at 37 °C did not cause significant changes in β-damascenone concentration, in contrast to what happens in beer. β-Damascenone production during heating under reflux was higher for beer samples than for wine samples, indicating that beer has more species capable of originating β-damascenone by heat treatment. β-Damascenone concentration almost doubled in wines acidified to pH 2, which is in agreement with the hypothesis that an acid hydrolysis is responsible for the formation/liberation of β-damascenone.

Acknowledgements

The authors thank UNICER—Bebidas de Portugal, SGPS S.A. for their support, including the supply of beer samples. This work was financed by Fundação para a Ciência e a Tecnologia (F.C.T.), as part of the Project POCTI 36453/QUI/2000.

References

Buttery, R. G., Teranishi, R., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry, 38*, 336–340.

Chevance, F., Guyot-Declerck, C., Dupont, J., & Collin, S. (2002). Investigation of β-damascenone level in fresh and aged commercial beers. *Journal of Agricultural and Food Chemistry, 50*, 3818–3821.

Demole, E., Enggist, P., Säuberli, U., Stoll, M., & Kovats, E. (1970). Structure and synthesis of damascenone, odorous constituent of Bulgarian rose oil (Rosa damascena). *Helvetica Chimica Acta, 53*, 541–551.

Gijs, L., Chevance, F., Jerkovic, V., & Collin, S. (2002). How low pH can intensify β-damascenone and dimethyl trisulfide production through beer aging. *Journal of Agricultural and Food Chemistry, 50*, 5612–5616.

Guido, L. F., Carneiro, J. R., Santos, J. R., Almeida, P. J., Rodrigues, J. A., & Barros, A. A. (2004). Simultaneous determination of *E*-2-nonenal and β-damascenone in beer by reversed-phase liquid chromatography with UV detection. *Journal of Chromatography A, 1032*, 17–22.

Isoe, S., Katsumura, S., & Sakan, T. (1973). The synthesis of damascenone and β-damascone and the possible mechanism of their formation from carotenoids. *Helvetica Chimica Acta, 56*, 1514–1516.

Kotseridis, Y., Baumes, R., & Skouroumounis, G. (1999). Quantitative determination of free and hydrolytically liberated β-damascenone in red grapes and wines using a stable isotope dilution assay. *Journal of Chromatography A, 849*, 245–254.

Kumazawa, K., & Masuda, H. (2001). Change in the flavor of black tea drink during heat processing. *Journal of Agricultural and Food Chemistry, 49*, 3304–3309.

Miller, J. C., & Miller, J. N. (1998). *Statistics for analytical chemistry* (second ed.). Chichester: Ellis Horwood Limited.

Puglisi, C. J., Elsey, G. M., Prager, R. H., Skouroumounis, G. K., & Sefton, M. A. (2001). Identification of a precursor to naturally occurring β-damascenone. *Tetrahedron Letters, 42*, 6937–6939.

Santos, J. R., Carneiro, J. R., Guido, L. F., Almeida, P. J., Rodrigues, J. A., & Barros, A. A. (2003). Determination of *E*-2-nonenal by high-performance liquid chromatography with UV detection: An assay for the evaluation of beer ageing. *Journal of Chromatography A, 985*, 395–402.

Skouroumounis, G. K., & Sefton, M. A. (2000). Acid-catalyzed hydrolysis of alcohols and their β-D-glucopyranosides. *Journal of Agricultural and Food Chemistry, 48*, 2033–2039.