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Volatile composition of Baga red wine Assessment of the identification of the would-be impact odourants

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Abstract

Wines produced from Baga native variety from the Portuguese Bairrada Appellation, harvest 2000, were submitted to a liquid–liquid continuous extraction with dichloromethane and analysis by gas chromatography–mass spectrometry (GC–MS). A total of 53 compounds were identified and quantified. This wine has 225 mg l^{-1} volatile compounds, which include aliphatic and aromatic alcohols (44%), acids (27%), esters (15%), lactones (6%), amides (5%), and phenols (1%). To achieve the identification of the major would-be impact odourants, the aroma index was calculated using the concentration of each volatile component and the corresponding odour threshold reported in the literature. This methodology proved suitable, as a preliminary step, for the determination of the would-be impact odourants of Baga wine. From the 53 compounds identified, nine were determined as the most powerful odourants: guaiacol, 3-methylbutanoic acid, 4-ethoxycarbonyl-γ-butyrolactone, *iso*butyric acid, 2-phenylethanol, γ-nonalactone, octanoic acid, ethyl octanoate and 4-(1-hydroxyethyl)-γ-butyrolactone. These data suggest Baga wine as a fruity-type product with an aroma correlated to a restricted number of compounds.

Keywords: Baga variety; Red wine; Volatile components; Aroma index; Would-be impact odourants

1. Introduction

Aroma substances are important in wine as they make a major contribution to the quality of the final product. The aroma of wines is the product of a biochemical and technological sequence (grape destemming, crushing, and pressing technology), and it is decisively influenced by the fermentation procedure [1]. All of these parameters determine the complexity of wine aroma. Alcohols, esters, acids, aldehydes, ketones, lactones, terpenoids and phenols, representing more than 800 volatile compounds, have been identified in grapes and wines [2–4]. The contribution of these compounds forms the olfactory character of a wine.

The grape and the winemaker in large measure determine wine quality. Fruit characteristics are governed principally by *terroir*, the combination of soil and climate, which influences grape composition and, subsequently, the wine quality. Overlaid on the basic quality derived from the grape is the mark of the winemaker. A skilful winemaker adjusts product variables in such a way that emphasizes one or more aromas, flavours, or tastes to produce a well-balanced and integrated product. Since many viticultural and enological factors greatly influence the types and concentrations of flavour components [4], the ability to determine each individual component would provide an approach to optimise the operational conditions (i.e. harvest parameters, juice preparation and fermentation techniques, use of yeasts, lactic acid bacteria and enzymes, and wine aging).

Particular attention has been devoted recently to the analytical characterization and the quality improvement of the varietal aroma of wines. Several studies have focused on the identification of volatile components of different varieties [5–7] and on the establishment of character impact odourants, which could contribute individually to the overall aroma of each variety [2,8,9]. Furthermore, studies on the identification of impact odourants associated to a particular varietal aroma have also been reported [10–13]. These studies concerning the establishment of character impact odourants always comprise gas chromography–olfactometric studies and/or the use of a sensory panel, which are time-consuming methodologies.

Baga is the main grape variety in Bairrada Appellation, an ancient winemaking region in Portugal. This variety

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represents 92% of the red vineyard, and 80% of the overall Bairrada vineyard, covering 15,000 ha, with a mean wine production of 450,000 hl. As a rule, Baga produces astringent and acidic young wines. Wine maturation, producing less astringent wines, matches the consumer's preferences. Because of its associated cost, the economic agents in Bairrada are discussing new technological approaches for Baga wines. Only the knowledge of the chemical composition of this variety can give opportunities for the adaptation of the characteristics of this old variety to new winemaking procedures ruled by the consumer's preferences. As far as we know, the aroma of this variety has not yet been characterized. In this research, attention was focused principally on the quantification of the volatile components of the Baga monovarietal wine in order to offer a means of evaluating the potential aroma of this variety and to improve the promotion of Baga wines.

2. Experimental

2.1. Materials

Vitis vinifera var., exclusively Baga grapes, from the 2000 harvest were collected in the Bairrada Appellation and monovarietal wine production was carried out in the Estação Vitivinícola da Bairrada, Anadia. The wine was prepared with a maceration step of 7 days at 28 °C. The musts were inoculated with active dry yeast (*S. cerevisiae* var. *cerevisiae* F10, Zymaflor F10), and the fermentations occurred, in triplicate, at 20 °C in 50 l microvinificators, with 40 kg of smashed grapes, with punching down twice a day. The fermentation was finished in 20 l glass vessels and, after fermentation, the wines were racked several times and, finally, were transferred to 0.75 l bottles. The bottles were stopped and stored at 15–20 °C until analysis, 6 months after fermentation.

The young Baga wine was described by the Bairrada winemakers as exhibiting an intense and very complex odour, characterized as a fruity-type product, presenting very ripe red fruits, cherry, red plum, strawberry, fig, pomegranate, blackberries, vegetable, and smoke/phenolic notes. The wine was also characterized as acidic, astringent and full-bodied. Baga wine does not exhibit any terpenic character.

2.2. Liquid–liquid continuous extraction

The extraction procedure was a modification of the method described by Etievant [14]. The wines were submitted to a process of liquid–liquid continuous extraction with dichloromethane. Five independent extractions were done. The wine (250 ml), supplemented with internal standard ethanolic solution (1.67 μg of 3-octanol), and 75 ml of dichloromethane were placed in the extractor. Extractions were carried out for 24 h at ca. 50 °C. The dichloromethane extracts were cooled to −20 °C to separate the frozen water from the organic phase by decantation and then dried over anhydrous sodium sulphate. The excess of low-boiling solvent was removed by distillation at low pressure using a trap with liquid nitrogen. The concentrate (about 1 ml) was stored in a glass screw-top vial at −20 °C.

2.3. Gas chromatography–mass spectrometry (GC–MS)

The extracts were analysed by GC–MS on a Hewlett-Packard 5890 series II gas chromatograph, equipped with a 30 m × 0.32 mm (i.d.), 0.25 μm coating thickness DB-FFAP fused silica capillary column, connected to a Hewlett-Packard Mass Selective Detector, according to the method described by Rocha et al. [5]. Splitless injections were used. The oven temperature was programmed from 35 to 220 °C at 2 °C min^{-1}, the injector temperature was 255 °C and the transfer line was heated at 250 °C. Helium carrier gas had a flow of 1.7 ml min^{-1} column head pressure of 12 psi. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV, scanning the range *m*/*z* 30–300 in a 1 s cycle. Identification of volatile compounds was achieved by comparison of the GC retention times and mass spectra with those, when available, of the pure standard compounds. All mass spectra were also compared with those of the data system library of the GC–MS equipment (Wiley 275) and other published spectra (Eight Peak Index of Mass Spectra, 1974, 2nd Ed., The Mass Spectra Data Centre, Nottingham, UK). Estimated concentrations for all compounds were made by peak area comparisons with the area of a known amount of internal standard (3-octanol). All of the five extracts were injected twice. The reproducibility of the result for the extracts was expressed as coefficient of variation (CV) in Table 1.

3. Results and discussion

3.1. Identification of volatile components

The volatile composition of the monovarietal Baga red wine is shown in Table 1. A total of 53 compounds were identified and quantified. The wine had 225 mg l^{-1} volatile compounds, which included aliphatic and aromatic alcohols (44%), acids (27%), esters (15%), lactones (6%), amides (5%), and phenols (1%).

Alcohols are quantitatively the largest group of the volatile compounds in Baga wine. The group is composed of aliphatic and aromatic alcohols, which include 2-heptanol, 1-hexanol, 2-nonanol, 2,3-butane diol isomers, methionol, benzyl alcohol and 2-phenylethanol. The alcohols occur in varying amounts, and they can be recognized by their strong and pungent smell and taste [15]. Most of these compounds were products of yeast fermentation [2]. The origin of 1-hexanol was reported as being related mainly to the lipooxygenase activity of the grape [16] and/or must aeration [17].

Table 1
Volatile components identified in dichloromethane extracts of Baga wines, grouped by chemical classes

RT		Identity[a]	Concentration ($mg\,l^{-1}$)[b]	CV (%)	Odour descriptor[c]	Odour threshold[c] ($mg\,l^{-1}$)
Alcohols						
11.7	2-Heptanol	A, B, C	0.27	15	Earthy, oily	–
13.2	1-Hexanol	A, B, C	4.82	10	Herbaceous	4.80
22.4	2-Nonanol	A, B, C	0.12	5	Fatty, mild, green, melon	–
23.6	(*R*,*R*)- + (*S*,*S*)-2,3-Butane diol	A, B, C	17.91	6	–	–
25.6	(*R*,*S*)-2,3-Butane diol	A, B, C	6.52	12	–	–
32.5	Methionol	A, B, C	2.31	12	–	–
40.8	Benzyl alcohol	A, B, C	0.71	12	Flowery-sweet	–
42.7	2-Phenylethanol	A, B, C	65.33	4	Flowery, rose, honey	10.0
	Subtotal ($mg\,l^{-1}$)		97.99			
	Subtotal (%)		43.6			
Acids						
17.5	Acetic acid	A, B, C	15.04	2	Vinegar	–
22.9	Propanoic acid	A, B, C	0.18	9	Fruity, acid, soapy-sweet	–
24.5	*iso*Butyric acid	A, B, C	2.63	3	Fruity, cheesy, sweaty	0.40
27.8	Butyric acid	A, B, C	0.23	10	Fatty-rancid, cheesy, sweaty	0.40
30.0	3-Methylbutanoic acid	B, C	1.95	6	Fatty-rancid, cheesy	0.25
39.5	Hexanoic acid	A, B, C	1.93	13	Rancid, grass, fruity	6.70
50.2	Octanoic acid	A, B, C	4.21	9	Fatty acid, dry, dairy	2.20
59.9	Decanoic acid	A, B, C	1.23	2	Fatty acid, dry, woody	1.40
64.8	Unknown acid (*m*/*z* 60)	–	31.20	5	–	–
71.2	Phenylacetic acid	A, B, C	0.84	12	Honey, pungent, floral	–
	Subtotal ($mg\,l^{-1}$)		59.44			
	Subtotal (%)		26.5			
Esters						
12.7	Ethyl 2-hydroxypropanoate	A, B, C	20.57	10	–	–
17.2	Ethyl octanoate	A, B, C	0.34	8	Ripe fruits, pear, sweety	0.24
21.6	Ethyl 3-hydroxybutyrate	B, C	0.58	14	Fruity	–
28.4	Ethyl decanoate	A, B, C	0.25	11	Sweety, fruity, dry fruits	1.10
30.8	Diethyl succinate	A, B, C	1.48	2	Cheese, earthy, spicy	–
35.9	Acetate ester (*m*/*z* 43, 88, 132)	B	0.14	14	–	–
37.4	2-Phenylethyl acetate	A, B, C	0.17	9	Fruity, floral, rose	0.25
37.8	Ethyl 4-hydroxybutanoate	B, C	6.61	4	–	–
49.4	Diethyl malate	A, B, C	0.81	10	Fruity	–
54.9	Diethyl 2-hydroxypentanodioate	B	0.43	6	–	–
59.1	Ethyl 2-hydroxy-3-phenylpropanoate	B	1.17	7	–	–
74.2	Ethyl vanillate	B, C	0.46	8	Vanilla, chocolate	–
	Subtotal ($mg\,l^{-1}$)		33.01			
	Subtotal (%)		14.7			
Lactones						
26.9	γ-Butyrolactone	A, B, C	6.13	14	Sweety, buttery	–
31.9	3-Methyl-2(5*H*)-furanone	A, B, C	0.34	6	Caramel	–
35.2	Tetrahydro-2*H*-pyran-2-one	B, C	0.18	9	Caramel	–
35.9	dihydro-4-methyl-2(3*H*)-furanone	B, C	0.14	11	Caramel	–
47.5	γ-Nonalactone	A, B, C	0.13	2	Coconut, fruity, almond-like	0.065
48.2	Pentolactone	B, C	0.53	15	–	1.60
49.7	5-Oxo-γ-hexalactone	B, C	0.46	6	Alcoholic	1.60
57.6	4-ethoxycarbonyl-γ-butyrolactone	B, C	2.72	14	Red fruits, cherry	0.40
61.4	4-(1-Hydroxyethyl)-γ-butyrolactone	B, C	1.29	13	Red fruits	1.60
63.8	4-(1-Hydroxyethyl)-γ-butyrolactone	B, C	2.12	8	Red fruits	1.60
70.8	Tetrahydro-4-hydroxy-4-methyl-2*H*-pyran-2-one	B, C	0.37	9	Caramel	–
	Subtotal ($mg\,l^{-1}$)		14.41			
	Subtotal (%)		6.4			
Amides						
29.2	*N*-ethylacetamide	B, C	0.59	12	–	–
41.8	*N*-(3-methylbutyl)acetamide	B, C	7.57	9	–	–

Table 1 (*Continued*)

RT		Identity[a]	Concentration ($mg\,l^{-1}$)[b]	CV (%)	Odour descriptor[c]	Odour threshold[c] ($mg\,l^{-1}$)
72.5	*N*-(2-phenylethyl)acetamide	B, C	2.15	8	–	–
	Subtotal ($mg\,l^{-1}$)		10.31			
	Subtotal (%)		4.6			
Phenols						
39.9	2-Methoxyphenol (guaiacol)	A, B, C	1.45	1	Smoky, burning, sweet, phenolic	0.075
47.5	Phenol	A, B, C	0.13	15	Phenolic	25.00
56.2	2-Methoxy-4-vinylphenol	A, B, C	0.35	8	Black pepper, species, clove-like	0.38
	Subtotal ($mg\,l^{-1}$)		1.93			
	Subtotal (%)		0.9			
Others						
9.5	3-Hydroxy-2-butanone	A, B, C	5.35	15	Buttery	–
20.4	*cis*-5-Hydroxy-2-methyl-1,3-dioxane	B, C	0.56	5	–	–
30.3	*trans*-4-Hydroxymethyl-2-methyl-1,3-dioxolane	B	0.36	16	–	–
38.4	*trans*- 5-Hydroxy-2-methyl-1,3-dioxane	B, C	0.26	1	–	–
46.7	Unknown (*m*/*z* 112, 55, 84)	–	0.44	8	–	–
51.4	3-Hydroxy-2-methyl-4*H*-pyran-4-one	B, C	0.16	13	Caramel	1.60
69.4	5-Hydroxymethylfurfural	A, B, C	0.17	9	Sweet, caramel	–
73.5	2-Ethoxycarbonyl-5-oxo-pyrrolidine	B	0.18	8	–	–
	Subtotal ($mg\,l^{-1}$)		7.48			
	Total ($mg\,l^{-1}$)		224.57			

[a] The reliability of the identification or structural proposal is indicated by the following: (A) mass spectrum and retention time consistent with those of an authentic standard; (B) structural proposals are given on the basis of mass spectral data (Wiley 275); (C) mass spectrum consistent with spectra found in the literature.

[b] Mean of 10 extraction replicates.

[c] Odour descriptor and odour threshold reported in the literature [1,18,25,27,31–35].

The fatty acids, formed enzymatically during fermentation, constitute an important group of aroma compounds that can contribute with fruity, cheese, fatty, and rancid notes to the wine's sensory properties [2,6]. Aliphatic acids (C_2–C_6, C_8, and C_{10}) and phenylacetic acid were identified.

In terms of the numbers of components identified, esters and lactones represent the largest groups, each with 12 individual compounds. In the case of esters, many were ethyl esters, although diethyl and phenylethyl esters were also detected. These esters are formed primarily during the fermentation [18,19] and are a characteristic of young wines.

Eleven γ-lactones (C_4–C_9) were identified with predominance of C_6. The most abundant lactone is γ-butyrolactone (44%). Every fermented product seems to contain γ-butyrolactone in which it probably arises from glutamic acid or related compounds [20]. The lactones are formed from the corresponding hydroxy acids [21]. The lactone content is dependent of the wine ageing and yeast strain [22]. Among the many volatile components of Baga wine, the lactones, particularly γ-lactones, seem to occupy a place of prominence in terms of their contribution to the total aroma and overall bouquet. The sensorial contributions of the different lactones are highly dependent on the chemical structure [23,20]. It is known that the type of ring (γ or δ), the functional groups and the length of the side chains, have an influence in the odour descriptor associated with the different lactones. In the homologous series of γ- and δ-lactones, the odour threshold decreases with increasing molecular weight [27].

Phenols present in young wines arise from the grapes and from the yeast metabolism [24]. These compounds may contribute to the overall aroma and form the body of the wine [15]. Furthermore, the volatile composition of Baga red wine comprises also the group of amides and other compounds not included in the groups described. Since these classes of compounds were not reported to have an influence on the sensorial properties of the wines, no particular attention was focused on them.

3.2. Purposes for the identification of would-be impact odourants

Of all the volatile compounds, only a limited number are important for aroma. Compounds that are considered as aroma contributing substances are primarily those present in concentrations higher than their odour threshold [27]. To achieve the identification of the would-be impact odourants, the concentration of each volatile component was related with the corresponding odour threshold reported in the literature (Table 1). For this purpose, the aroma potential of each compound was assessed by calculating the aroma index (I) using the equation $I = c/s$ [25], where c is the

concentration in the wine and s is the olfactory perception threshold in wine, in an aqueous alcohol solution or in water, depending to the information available in the literature. On the basis of their odour description and threshold, the most powerful odourants of Baga wines were tentatively established. Compounds that exhibit $I > 1$ were considered to contribute individually to the wine aroma and were designated as would-be impact odourants. Based on the fact that the influence of the matrix was low on the descriptors of the aroma perceived but was high on their intensity [26], and because the values of the odour threshold reported in Table 1 were not determined in the Baga wines, they can be used only to establish the would-be impact odourants of this monovarietal wine. Furthermore, according to Meilgaard's suggestion of the sensorial contribution to the overall aroma of a substance, when its concentration is at least 20% of the threshold unit ($I > 0.2$), it should be considered [6,27].

3.3. Assessment to the identification of would-be impact odourants

From an organoleptic point of view, natural lactones are generally characterized by a fruity odour. Among the many volatile components of wine, lactones, particularly γ-lactones, occupy a place of prominence in terms of their contribution to the overall aroma [20]. γ-Butyrolactone always occurs in wines [20], and it is associated with a buttery [27] and rubber descriptor [28]. In this Baga wine its concentration was $6.13\,mg\,l^{-1}$. However, the absence of odour threshold values for this compound prevents knowledge of its odour contribution. The pentolactone, the 5-oxo-γ-hexalactone and the isomers of the 4-(1-hydroxyethyl)-γ-butyrolactone are γ-C6 lactones, and according to Dufossé et al. [23] possess a hot and herbaceous odour. Of these, only the isomers of the 4-(1-hydroxyethyl)-γ-butyrolactone exhibit an aroma index higher than 1 ($I = 1.3$) and, therefore, can contribute for Baga aroma. γ-C6 lactones are associated with red fruits aroma, such as strawberry, cherry and raspberry [23]. The concentration of 4-ethoxycarbonyl-γ-butyrolactone ($2.72\,mg\,l^{-1}$) is significant within its limit of perception ($0.40\,mg\,l^{-1}$). The aroma index of 6.8 suggests that this lactone is present in wine at a level of sensorial contribution. This lactone may contribute with its cherry aroma [20]

to their aroma index and odour descriptor, they seem to be relevant to the aroma of Baga wine, confirming it as a fruity-type product presenting ripe red fruits, cherry, strawberry, vegetable, sweet and smoke/phenolic notes, which are closely related to the aroma of young Baga wine described by the Bairrada winemakers. Some of these compounds have already been reported as impact odourants of several wine varieties [2,8,9], but this set of compounds had not yet been reported for a monovarietal wine.

The proposed methodology, which does not include the use a sensory panel and/or GC–olfactometric studies, seems to be adequate, as a preliminary step, to establish the would-be impact odourants of Baga wines. However, further steps, such as GC–olfactometric studies are necessary to confirm the impact of the odour-active compounds already identified.

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