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FIRST EVALUATION OF A TRADITIONAL PORTUGUESE ALCOHOLIC BEVERAGE, PREPARED WITH MACERATION OF JUNIPER BERRIES

CARACTERIZAÇÃO DE UMA BEBIDA ALCOÓLICA TRADICIONAL PORTUGUESA, PREPARADA COM MACERAÇÃO DE BAGAS DE ZIMBRO

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SUMMARY

The alcoholic drink called 'aguardente de zimbro' is a traditional Portuguese alcoholic beverage made by the people living around Serra da Estrela, using the maceration of juniper berries in different distillates like arbutus spirit, marc spirit and wine spirit. Traditionally homemade for own consumption in rural families, this beverage is now sold to tourists and promoted and included in the food patrimony of the region. However, there is a lack of technical and scientific information about this beverage. A first evaluation of this beverage is done in this work, based on its sensory and physicochemical characteristics. A few commercial samples, two homemade and another two samples of alcoholic beverages made with juniper were analysed in this work. Several analytical determinations, namely density, alcoholic strength, dry matter, total acidity, fixed acidity, volatile acidity, pH, chromatic characteristics, methanol, acetaldehyde, ethyl acetate, fusel alcohols and total antioxidant activity were done on the samples with the aim of characterising these Portuguese flavoured juniper spirits. The descriptive sensory analysis of these drinks was also done in order to find the more characteristic sensory attributes of the drink. The results show that under the label 'aguardente de zimbro' there are several drinks, with a yellow colour, but with several differences in physicochemical composition and sensory description. In fact, these drinks, with ethanol content from 37.7 to 48.3 % vol. present values of acidity from 25 to 1360 mg/L, pH from 3.91 to 5.89, dry matter 0.75 to 75.24 g/L and density from 0.9446 to 0.9770 g/cm^3. Important differences were also observed in the chromatic characteristics, volatile composition and total antioxidant activity. Therefore, further research must be done in order to characterise this 'traditional' drink.

RESUMO

A bebida alcoólica denominada "aguardente de zimbro" é uma bebida alcoólica tradicional portuguesa produzida na região da Serra da Estrela, elaborada a partir da maceração de bagas de zimbro em diferentes destilados nomeadamente aguardente bagaceira, aguardente vínica e aguardente de medronho. Esta bebida, que era tradicionalmente produzida para consumo próprio nas famílias rurais, é atualmente produzida industrialmente e vendida aos turistas como uma das imagens de marca dos produtos tradicionais da região. No entanto, há uma falta de informação técnica e científica sobre esta bebida. Neste trabalho é efetuada uma primeira caracterização desta bebida com base nas suas características sensoriais e físico-químicas. Neste trabalho foram analisadas algumas amostras comerciais, duas amostras caseiras e duas amostras de outras bebidas alcoólicas feitas com zimbro. Foram realizadas as seguintes determinações analíticas nas diferentes amostras recolhidas: densidade, teor alcoólico, matéria seca, acidez total, acidez fixa, acidez volátil, pH, características cromáticas, metanol, acetaldeído, acetato de etilo, álcoois superiores e atividade antioxidante total. Foi efetuada também, a caracterização sensorial descritiva dessas bebidas a fim de encontrar os atributos sensoriais mais característicos da bebida. Os resultados mostram que, sob a designação de "aguardente de zimbro" existem várias bebidas, com uma cor amarela, mas com algumas diferenças na composição físico-química e descrição sensorial. Efetivamente, estas bebidas, com um teor de etanol entre 37.7 e 48.3 % vol., apresentam valores de acidez de 25 a 1360 mg/L, pH de 3.91 a 5.89, matéria seca de 0.75 a 75.24 g/L e densidade de 0.9446 a 0.9770 g/cm^3. Foram também verificadas diferenças importantes nas características cromáticas, composição volátil e atividade antioxidante total. É de referir, no entanto, que são necessários mais estudos de modo a aprofundar a caracterização desta bebida "tradicional".

Key words: 'Aguardente de zimbro', juniper, spirit; descriptive sensory analysis; physicochemical characterisation.
Palavras-chave: Aguardente de zimbro, zimbro, aguardente, análise sensorial descritiva, caracterização físico-química.

INTRODUCTION

Traditional foods are an important element of cultural heritage and play an important role in local identity and consumer behaviour. In addition the traditional foods are very important for rural development and for the sustainability of small and medium sized enterprises (Albayrak and Gunes, 2010).

Juniperus communis L. is a gymnosperm that belongs to the Cupressaceae family usually called juniper. It is a shrub or small tree distributed in Mediterranean mountains, up to the subarctic tundra, that produces berries (female cones) containing a small number of seeds known for their physiological properties (Angioni *et al.*, 2003; Barjaktarovic et al., 2005;Orav *et al.*, 2010). In northern and central Europe this species is common, but in the Mediterranean Basin its distribution becomes more fragmented and the species are located exclusively in high-mountain areas (García *et al.*, 2000).

J. communis has shown antimicrobial and antibacterial activity mainly due to its richness in phenolic and terpenic compounds and organic acids (Gordien et al., 2009; Martz *et al.*, 2009; Carpenter *et al.*, 2012). Several studies done on juniper berries established their composition in essential oils (Orav *et al.*, 2010; Robbat *et al.*, 2011), resins, fats, sugars and dyes and vitamin C (Angioni *et al.*, 2003; Shahmir *et al.*, 2003; Barjaktarovic *et al.*, 2005; Haziri *et al.*, 2013).

The Juniper berries are also used for medicinal purposes and as diuretics to treat some diseases, such as urinary infections, kidney problems, stomach acid, tuberculosis, digestion disorders including colic and for external use it is used to treat rheumatism and arthritis (Newton *et al.*, 2002; Allen e Hatfield, 2004; Orhan *et al.*, 2012).

Juniper berries are well known as a flavouring agent in foods and alcoholic beverages. These berries are used for flavouring gin and in the production of juniper and juniper-flavoured spirit distillates (McDonnell *et al.*, 2001; Vichi *et al.*, 2007).

According to European legislation (Regulation EC No 110/2008) the juniper berries can be used in the production of several alcoholic beverages namely juniper spirit, juniper-flavoured spirit drink, gin, distilled gin, London gin and Berenburg:

1. **Juniper spirit** is a fruit spirit, a brandy produced exclusively by alcoholic fermentation and distillation of fleshy fruit or must of such fruit, berries or vegetables, with or without stones.

2. **Juniper-flavoured spirit drink** is a drink produced by flavouring ethyl alcohol of agricultural origin and/or grain spirit and/or grain distillate with juniper (*J. communis* L. and/or *Juniperus oxicedrus*).

3. **Gin** is a juniper-flavoured spirit drink produced by flavouring organoleptically suitable ethyl alcohol of agricultural origin with juniper berries (*J. communis* L.).

4. **Distilled gin** is: (i) a juniper-flavoured spirit drink produced exclusively by redistilling organoleptically suitable ethyl alcohol of agricultural origin of an appropriate quality with an initial alcoholic strength of at least 96 % vol. in stills traditionally used for gin, in the presence of juniper berries (*J. communis* L.) and of other natural botanicals provided that the juniper taste is predominant, or (ii) the mixture of the product of such distillation and ethyl alcohol of agricultural origin with the same composition, purity and alcoholic strength; natural and/or nature-identical flavouring substances and/or flavouring preparations may also be used to flavour distilled gin.

5. **London gin** is a type of distilled gin: (i) obtained exclusively from ethyl alcohol of agricultural origin, with a maximum methanol content of 5 grams per hectolitre of 100% vol. alcohol, its flavour is introduced exclusively through the redistillation in traditional stills of ethyl alcohol in the presence of all the natural plant materials used, (ii) the resultant distillate which contains at least 70 % alcohol by vol., (iii) where any further ethyl alcohol of agricultural origin is added it must be consistent with appropriate characteristics, but with a maximum methanol content of 5 grams per hectolitre of 100 % vol. alcohol.

6. **Berenburg ou Beerenburg** is a spirit drink produced from ethyl alcohol of agricultural origin, macerated with fruit or herbs or parts of plants or fruits, containing as specific flavour distillate of gentian root (*Gentiana lutea* L.), of juniper berries (*J. Communis* L.) and of laurel leaves (*Laurus nobilis* L.).

In Portugal, junipers have the name 'Zimbro' and they grow and are very characteristics in 'Serra da Estrela' and 'Serra do Gerês' (Costa *et al*., 1998). Their fruits are used to produce a traditional distillate flavoured with Juniper which is obtained by macerating the juniper berries into a distillate that could be grape marc spirit, arbutus spirit or wine spirit. These beverages were traditionally homemade, for own consumption of rural families. However, as a result of tourism increasing in "Serra da Estrela", a tourist region of Portugal, some commercial flavoured juniper beverages appeared on the market and are sold to tourists. Nevertheless, there is no scientific data on the chemical and sensory composition of these alcoholic beverages. Thus this study aims to produce a first characterisation of this product concerning its physicochemical and sensory characteristics.

MATERIAL AND METHODS

Samples

Three bottles of each of the different brands of Portuguese juniper flavoured beverages were purchased at the market. Only three different products, which were coded as Z1, Z2 and Z3, were found at the Portuguese market. In order to compare with juniper alcoholic beverages other samples from other countries namely a London Dry Gin and a Gin from Mahon were also purchased, which were coded as Z4 and Z5.

An attempt was made to find some homemade samples but only two samples were obtained (about of 500 mL of each sample) which were kindly given by two artisanal producers from the same region of the commercial samples, and produced with grape mark spirit. These samples were coded as Z6 and Z7.

All the Portuguese samples present a few junipers berries inside the bottles.

Based on the label information and on information given by the producers Z1 sample is produced with a spirit flavoured with juniper berries; Z2 is produced with arbutus distillate and flavoured with juniper berries and vanilla; Z3 sample is produced with grape marc spirit flavoured with juniper berries and aged in wooden oak barrels. No information about the

quantity of Juniper berries used in the process or the macerating time was available for the commercial or artisanal beverages.

Reagents

Ethanol and methanol was purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) was purchased from Aldrich (Steinheim, Germany).

GC-FID standards: Ethyl acetate [CAS Nº 141-78-6; purity ≥99.8%] was purchased from Riedel-de-Haen (Seelze, Germany), methanol [CAS Nº 67-56-1; purity ≥99.9%] was purchased from Merck (Darmstadt, Germany). 2-methyl-1-butanol [CAS Nº 34713-94-5; purity ≥98%] 3-methyl-1-butanol [CAS Nº 123-51-3; purity ≥98.5%], 1-butanol [CAS Nº 71-36-3; purity ≥99.5%], 2-methyl-1-propanol [CAS Nº 78-83-1; purity ≥99.5%], 1-propanol [CAS Nº 71-23-8; purity ≥99.5%], 2-propen-1-ol [CAS Nº 107-18-6; purity ≥98%], 2-butanol [CAS Nº 78-92-2; purity ≥99.5%], 4-methyl-2-pentanol [CAS Nº 108-11-2; purity ≥97%] and acetaldehyde [CAS Nº 75-07-0; purity ≥99.5%] were purchased from Fluka (Buchs, Switzerland).

Distilled water was used to prepare the hydro alcoholic solutions.

Analytical determinations

In order to characterise the alcoholic drinks, some analytical determinations are done on all the samples: alcoholic strength, dry matter, total acidity, fixed acidity, volatile acidity, pH, chromatic characteristics and methanol, acetaldehyde, fusel alcohols and ethyl acetate concentration.

Alcohol strength

The alcohol strength was determined by distillation and electronic densimetry (OIV, 2014), using an electronic densimeter Model 5000 DMA brand Anton Paar. The results are the arithmetic mean of the two replicate analysis and they are present as volumetric percentage of ethanol in the beverage.

Mass density

The mass density was evaluated by electronic densimetry (OIV, 2014) according to the method recommended for the spirits, using a densimeter Model 5000 DMA brand Anton Paar.

Dry extract

The dry extract is evaluated by the method suggested for spirits by OIV (2014), which consists in weighing the residue left by evaporation, at 100 °C, of spirits. The results are expressed in g per litre.

Total acidity

Total acidity was determined by titrimetry (Belchior and Carvalho, 1984). The results are expressed in mg of acetic acid per litre.

Fixed acidity

Fixed acidity was determined by titrimetry on the water solution of dry extract (Belchior and Carvalho, 1984). The results are expressed in mg of acetic acid per litre.

Volatile acidity

Volatile acidity was determined by calculation (total acidity minus fixed acidity) (Belchior and Carvalho, 1984; OIV, 2014). The results are expressed mg of acetic acid per litre.

pH

The pH was determined by using a potentiometer Crison, according to the method from OIV (2014).

Chromatic characteristics

The chromatic characteristics were determined on a Varian Cary 100 Bio spectrophotometer (Palo Alto, USA) and a 10 mm glass cell, by measuring the transmittance of the sample every 10 nm from 380 to 770 nm, with a D65 illuminant. Based on the transmittance values some parameters were calculated: luminosity (L*); saturation (C*); chromaticity coordinates (a* and b*). The coordinate a* takes positive values for reddish colours and negative values for greenish ones, while coordinate b* takes positive values for yellowish colours and negative values for bluish ones (OIV, 2014).

Methanol, acetaldehyde, ethyl acetate and fusel alcohols

The volatile compounds of the samples were analysed by gas chromatography-flame ionization detection (GC-FID) according to the method of Luis *et al*. (2011).

Preparation of samples to GC-FID: The compounds, in the spirit drinks, are determined by direct injection of the distillate, obtained in the alcohol strength determination. Prior to injection 9 mL of each sample (distillate of spirit drink, standard solution) was added with 1 mL of internal standard solution (4-methyl-2-pentanol).

Analysis by GC-FID: GC-FID analysis was carried out using an Focus GC gas chromatograph (Thermo Scientific, USA) equipped with a flame ionisation detector-FID (250ºC) and a fused silica capillary column of polyethylene glycol (DB-WAX, JW Scientific, Folsom, CA, USA), 60 m length, 0.32 mm i.d., 0.25 µm film thickness. The carrier gas was hydrogen (3.40 $cm^3.min^{-1}$). The samples were injected (~1 µl) on the injector (200 ºC) in split mode (split ratio 1:6). The oven temperature program was 35 ºC (for 8 min), then increased at $10ºC.min^{-1}$ to 200 ºC and held at this temperature for a further 9 min (Luis *et al*. 2011). The results will be expressed in grams per hectolitre of 100 % vol. alcohol, which will be abbreviated as AE-absolute ethanol (g/hL AE).

Total antioxidant activity

The measurement of the total antioxidant activity was done according to the method described by Canas *et al.* (2008). 10 µl of sample was added to 3 ml of an 8.5 x 10^{-5} M DPPH methanol solution prepared as described in Canas *et al.* (2008). Pure methanol solution was used as a reference. The decrease in absorbance, measured at 515 nm, was determined continuously until the reaction reached a plateau, and the calculations are done according to the mentioned authors.

The total antioxidant activity was only determined in commercial juniper flavoured Portuguese beverages (Z1, Z2 and Z3).

Sensory analysis of alcoholic juniper drinks

The sensory analysis was done only on the samples Z1, Z2, Z3, Z4 and Z5. The low volume of Z6 and Z7 samples did not allow for sensory analysis.

10 trained panellists (7 women and 3 men with ages ranged from 24 until 59 years) were used for the sensory characterisation of these distillates. The tasting panel was composed of a group of tasters previously selected and trained for quantitative descriptive analysis of wine brandies (Caldeira *et al.*, 1999) and wines (Teixeira, 2011). The retraining sessions were performed with several aroma standards in hydroalcoholic solutions.

The samples were tasted in a sensory room, in balanced orders to eliminate first-order carry-over effects (Williams, 1949), presented as 30 mL to the panel in wine tasting glasses (ISO 3591, 2010), at room temperature and under white natural lighting. Water was provided for mouth rinsing between samples.

The tasters were asked to formulate a free description of colour, aroma and flavour of the five samples under evaluation. They were also asked to rate the similarity of the ten possible pairs of different five samples using a 5-point structured scale (1-very dissimilar to 5- very similar).

RESULTS AND DISCUSSION

According to the information collected from few artisan producers, who did not give any sample to this work, the traditional Portuguese flavoured juniper drink is obtained by the maceration of juniper berries in different alcoholic drinks (arbutus spirit, wine spirit or grape mark spirit). The macerating time and the quantity of the berries used are quite different ranged from 40 to 90 berries per litre and from any time until a year or more. So, further work must be done by inquiring more artisanal producers in order to collect more information about the traditional technological procedure. Nevertheless, it was not possible to obtain more information about the technology of analysed samples, only the information on the labels, which is presented at material and methods.

This beverage is traditionally called 'aguardente de zimbro' which means juniper spirit. However, according to the European regulation definitions (Regulation EC No 110/2008), which are presented in the introduction, these traditional Portuguese drinks are not juniper spirits because the fermentation of the junipers does not occur. Thus, these drinks should be included in the category of juniper flavoured spirit drinks but its legal definition must be improved in order to include other spirit drinks, such as arbutus spirit, mark spirit and wine spirit, in the maceration process.

Physicochemical analysis

Tables I to III show the results of the physicochemical determinations done on the juniper flavoured alcoholic drinks.

Table I

Results of the density, alcohol strength, total acidity, fixed acidity, volatile acidity, pH and dry matter of the samples studied, considering the average and the standard deviation of two determinations for each sample.

Resultados da densidade, teor alcoólico, acidez total, acidez fixa, acidez volátil, pH e matéria seca das amostras estudadas, considerando-se a média e o desvio-padrão de duas determinações para cada amostra.

		Sample code	Density (g/cm^3)	Alcohol strength (%vol.)	Total acidity (mg of acetic acid/L)	Fixed acidity (mg of acetic acid/L)	Volatile acidity (mg of acetic acid/L)	pH	Dry matter (g/L)
Portuguese juniper flavoured drinks	**Commercial samples**	Z_1	0.9492±0.0000	39.2±0.06	25±0.00	25±0.00	0±0.00	5.89±0.05	0.85±0.04
		Z_2	0.9493±0.0000	39.3±0.05	454±0.00	25±0.00	429±0.00	4.11±0.01	0.84±0.03
		Z_3	0.9446±0.0001	42.4±0.05	504±0.00	76±0.00	428±0.00	4.56±0.01	3.10±0.02
	Homemade samples	Z_6	0.9333±0.0000	48.3±0.08	1360±2.42	22±3.43	1338±0.00	3.91±0.07	0.75±0.06
		Z_7	0.9770±0.0003	37.7±0.13	441±3.43	136±3.43	305±0.00	4.67±0.02	75.24±0.23
	Range (minimum/maximum)		0.9446/0.9770	37.7/48.3	25/1360	25/136	0/1338	3.91/5.89	0.75/75.24
London gin		Z_4	0.9482±0.0001	39.9±0.05	25±0.00	13±0.00	12±0.00	6.27±0.06	0.06±0.00
Gin		Z_5	0.9515±0.0000	37.8±0.01	12±0.00	0±0.00	12±0.00	8.06±0.04	0.38±0.00

Table II

Results of the colour parameters of the samples studied, considering the average and the standard deviation of two determinations for each sample.

Resultados dos parâmetros de cor das amostras estudadas, considerando-se a média e o desvio padrão de duas determinações para cada amostra.

		Sample code	L*	a*	b*	C*
Portuguese juniper flavoured drinks	**Commercial samples**	Z_1	98.5	0.02	8.78	8.85
		Z_2	98.5	-1.76	10.43	10.58
		Z_3	91.2	-0.45	28.77	28.78
	Homemade samples	Z_6	98.1	-0.92	6.75	6.81
		Z_7	95.9	-1.48	8.35	8.68
	Range (minimum/maximum)		91.2/98.5	-1.76/0.02	6.75/28.77	6.81/28.78
London gin		Z_4	100.8	-0.04	-0.23	0.23
Gin		Z_5	100.1	-0.04	0.05	0.06

Table III

Results of methanol, acetaldehyde, ethyl acetate and some fusel alcohol of the samples studied, considering the average and standard deviation of two determinations for each sample.

Resultados de metanol, acetaldeído, acetato de etilo e alguns álcoois superiores das amostras estudadas, considerando-se a média e o desvio padrão de duas determinações para cada amostra.

Sample code	Methanol (g/hl AE)	Acetaldehyde (g/hl AE)	Ethyl acetate (g/hl AE)	1-Propanol (g/hl AE)	2-Methyl-1-propanol (g/hl AE)	2+3-Methyl-1-butanol (g/hl AE)
Z_1	<QL[1]	7.7 ± 0.3	<QL[3]	<QL[4]	<QL[5]	5.5 ± 0.22
Z_2	330 ±1.8	64.6 ± 7.2	112.3 ± 9.3	54.5 ± 0.24	83.7 ± 0.2	247.1 ± 1.2
Z_3	455 ± 6.1	140.3 ± 4.7	48.0 ± 5.1	39.6 ± 0.3	69.7 ± 0.7	276.7 ± 0.8
Z_6	200.1±3.3	28.61±1.8	356.5±7.8	53.9±0.5	89.4±0.9	331.1±3.2
Z_7	106.9±2.4	<QL[2]	< QL[3]	45.0±0.2	82.5±0.1	538.2±0.10
Range (minimum/maximum)	QL/455	QL/140.3	QL/356.5	QL/54.5	QL/89.4	5.5/538.2
Z_4	< QL[1]	<QL[2]	< QL[3]	< QL[4]	< QL[5]	<QL[6]
Z_5	< QL[1]	6.3 ± 1.12	< QL[3]	< QL[4]	< QL[5]	<QL[6]

Values below the quantification limit calculated by Luis et al (2011): 1- 3.51 g/hl AE; 2 - 4.92 g/hl AE; 3- 1.18 g/hl AE; 4 - 0.64 g/hl AE; 5- 0.60 g/hl AE; 6 - 0.35 g/hl AE.

All the Portuguese flavoured juniper beverages (commercial: Z1, Z2, Z3 and homemade samples: Z6, Z7) present the alcoholic strength values (Table I) above the minimum of 30% vol. required by European Regulation (Regulation EC No 110/2008). The chemical composition of these beverages (Table I) seems quite different, which could probably be due to the use of different kinds of spirits for macerating the juniper berries, the use of different amounts of juniper berries and the use of different maceration times.

The dry matter of some Juniper flavoured spirit drinks (Z1, Z2, and Z6) is very similar. However, for Z3 and Z7 samples the dry matter is high. For Z3 it is possible that the ageing in wood also contributes to the increase of dry extract, taking into account that the wine brandy wood ageing leads to an increase of dry matter (Belchior *et al*., 2001). For the Z7 it is possible to admit that the amount of junipers and/or the maceration time will be different, and that could explain the high levels of dry matter.

Samples Z4 and Z5 are those with lower values comparing the dry matter content, which would be expected since they are distilled beverages, where the maceration process with the fruit will have occurred before distillation.

In the traditional Portuguese samples (Z1, Z2, Z3, Z6 and Z7), the pH values are quite different (Table I), ranging from 3.91 to 5.89. The high value of pH was found on Z1 sample. These results suggest the use of different spirits in the maceration but it is also known that pH values also ranged in the same spirit beverage as shown in the grape mark spirit by Borsa et al. (2007) which found pH values ranging from 3.70 to 5.83 and in wine spirits by Henriques (1987) which found pH values ranging from 4.96 to 5.56.

The samples of London gin (Z4) and gin (Z5) present high values of pH.

In accordance with pH values the juniper flavoured drinks show different values of total acidity, and the low values are determined in Z1, Z4 and Z5 (Table I). Once again the differences in total acidity for Portuguese traditional Juniper flavoured spirit drinks suggest the use of different technologies, concerning the spirit drink used for the maceration and/or the use of different amounts of berries and different maceration times. In fact, the values of acidity of different spirits are quite different. Espírito Santo (2010) report for arbutus spirit values of total acidity between 0.10 and 0.19 g/L; Orriols *et al.* (2008) found acidity values from 0.37 to 0.53 g/L in grape mark spirits and Henriques (1987) found values from 0.10 to 0.27 g/L in wine spirits.

Like in other spirits (Puech *et al.*, 1984), the total acidity of the samples is mainly composed by the volatile acidity (Table I) with the exception of sample Z7, which presents the highest value of fixed acidity. This result suggests a more pronounced extraction process in the production of sample Z7.

The results of chromatic characteristics of the analysed samples are shown in Table II.

According to the method (OIV, 2014) the luminosity L* varies between 0 (black) and 100% (colourless); the values of a* and b* vary respectively between -a (green) to +a (red) and between -b (blue) to +b (yellow); the C* value corresponds to purity or colour saturation.

All the distillates are quite transparent. The Gin (Z5) and London gin (Z4) are completely transparent while the Portuguese juniper flavoured drinks are less transparent. The darker samples are Z3 and Z7 results. In the Z3 sample the dark colour could be due to the contribution of wood ageing but in Z7 sample this could be due a more pronounced maceration/ extraction process.

Concerning the colour component a*, it seems that the majority of the Portuguese samples tend to present a green colour with the exception of Z1 which presents a tendency to red. Based on the b* values all the Portuguese juniper flavoured beverages present a yellow colour, which is more pronounced in the commercial samples while the London gin (Z4) tends to present a blue colour.

Regarding the colour saturation C*, the values are also quite different and the highest value is detected in the Z3 sample.

Table III presents the results of the quantification of the volatile compounds analysed. The concentration of 2-butanol, 2-propen-1-ol and 1-butanol are not show in Table III because all the samples have values below the quantification limits which are respectively 1.11, 0.16 and 0.18 g/hL AE (Luis *et al.*, 2011). Only Z3 sample exhibits a concentration of 1.8 g/hL AE of 1-butanol.

Like in other spirits (Nykänen and Suomalainen, 1983; Luis *et al.*, 2011; Espírito Santo, 2010), the most abundant volatile compounds in these distilled spirits are the fusel alcohols, the ethyl acetate, together with acetaldehyde and methanol. The fusel alcohols, ethyl acetate and the acetaldehyde are mainly resulting from yeast and bacteria metabolism during the fermentation stage (Nykänen and Nykänen, 1991; Espírito Santo, 2010) while the methanol is derived from enzymatic degradation of fruit and pomace fruit pectins (Gnekow and Ough, 1976).

The traditional Portuguese Juniper-flavoured spirit drinks (Z1, Z2, Z3, Z6 and Z7) display a very different volatile composition. The values of these determinations are quite different, especially in the sample Z1. The minimum of volatile substances (the methanol are not included) of 125 g/hL, 140 g/hL and 200 g/hL AE required (Regulation EC No 110/2008) respectively to the wine spirit, marc spirit and arbutus spirit are not attained in sample Z1. Thus, this sample is probably obtained by using another spirit, different

from the traditional process, probably ethyl alcohol of agricultural origin, as used for the production of other flavoured juniper beverages. The similar volatile composition of Z1, Z4 and Z5 corroborate this hypothesis.

Because of its well know toxicity (Henderson and Brubacher, 2002), the methanol content is limited by law (Regulation EC No 110/2008 and Decreto-Lei nº 238/2000) at 1000 g/hL AE for grape marc spirits and arbutus spirit and at 200 g/hL AE for wine spirits.

All the samples present the methanol content below the legal limits. The highest value is found in the sample Z3 which is prepared with grape marc spirit, which tends to present high values in methanol (Mota *et al.*, 2010) owing to its technology.

The acetaldehyde is also present in the distillates and proceeds from the fermentation stage (Nykänen and Suomalainen, 1983) but its content could increase by the ethanol oxidation (Reazin *et al.*, 1976). The highest content is also found in the Z3 sample and corresponds to the usual range of values usually determined in grape mark spirits (Luis *et al.*, 2011).

The ethyl acetate, the main ethyl ester in several distillates (Nykänen and Suomalainen, 1983), increases its amount over the wood ageing time (Puech *et al.*, 1984) due to the esterification reactions which take place (Reazin *et al.,* 1976) and are favoured by the high contents of ethanol. The high amounts of ethyl acetate are found in samples Z2 and Z6. According to Galego (2006), the arbutus spirit presents higher values of ethyl acetate than the grape marc spirit or wine spirit, which is in accordance with the higher value found for the Z2 sample and supports the indication that this beverage was produced with arbutus spirit. However, the amounts of 1-propanol, 2 methyl-1 propanol and 2+3 methyl-1-butanol are above the maximum limits specified in Portuguese legislation (Dec-Lei nº 283/2000) for arbutus distillate. Thus, this sample (Z2) probably contains arbutus spirit and also other spirit with high amounts of fusel alcohols.

The fusel alcohols are an important group of alcohols present in spirits (Nykänen and Suomalainen, 1983) which contribute to the complexity of the flavour even after the wood ageing process (Caldeira *et al.*, 2008). The low contents of alcohols in samples, Z1, Z4, Z5 is certainly related to the use of a more rectified distillate, namely the ethyl alcohol of agricultural origin.

The sample Z7 presented low amount of acetaldehyde and the ethyl acetate and high amounts of 2+3 methyl-1-butanol. Taking in account that all of these compounds result from the microbial metabolism (Nykänen and Suomalainen, 1983) these results seem to suggest a quite different fermentation process.

Considering the knowledge on antioxidant activity of juniper berries (Elmastas *et al.*, 2006; Loizo *et al.*, 2007) the total antioxidant activity in commercial Portuguese flavoured juniper drinks was investigated (Table IV).

The total antioxidant activity of the Juniper-flavoured spirit drink is higher for samples Z2 and Z3. This activity is almost zero for the Z1 sample. These differences must be due to the differences in the conditions of maceration on juniper berries just as verified in beer (Veljovic *et al.*, 2011). The higher antioxidant activity of the Z3 sample could be also due to the contribution of wood compounds as demonstrated by Canas *et al.* (2008) with aged brandies. However, the antioxidant activity is lower than the values determined in wood aged wine brandies (Canas *et al.,* 2008).

Table IV

Average values of total antioxidant activity of the three commercial samples of Portuguese flavoured juniper drinks

Valores médios da atividade antioxidante total das três amostras comerciais de bebidas portuguesas aromatizadas com zimbro

	Sample code	Total antioxidant activity (% DPPH inhibition)
Commercial samples of Portuguese flavoured juniper drinks	**Z_1**	2.9±0.1
	Z_2	23.8±0.4
	Z_3	34.8±1.6

Sensory evaluation

The Tables V, VI and VII display the visual, olfactory and gustatory attributes of different distillates, flavoured with juniper, generated by the tasters, as well as the absolute frequency of the attribute for each sample.

Given the colour characteristics presented in Table V, samples Z4 and Z5 are clear and transparent, because they are drinks without aging or maceration after

the distillation, in accordance with the chromatic characteristics shown in Table II. The commercial Portuguese juniper flavoured drinks (Z1, Z2 and Z3) present yellow as the predominant colour that ranges from the yellow green to the golden colour that is consistent with the results of the chromatic characteristic in Table II. The Z3 is the only one with a topaz colour which could be related to the wood ageing which promotes the increase in the topaz colour in wine brandies (Canas *et al.*, 2000). The characteristic colour is certain a result of the maceration process with juniper berries after distillation. These samples are clear but Z2 and Z3 present particles in suspension that could result from the disruption of the juniper berries inside the bottle.

In the sensory evaluation the panel generated 24 odour attributes (Table V) and 17 flavour attributes (Table VI). The attributes like floral, fruity, spicy, citrus, eucalyptus, fresh, resin, sour and rose have

Table V

Visual attributes generated by the tasters to describe the colour and appearance of the samples. The numbers correspond to the absolute frequency of the attribute for each evaluated sample.

Atributos visuais gerados pelos provadores para descrever a cor e a aparência das amostras. Os números correspondem à frequência absoluta do atributo para cada amostra analisada.

		Portuguese juniper flavoured drinks (commercial samples)			**London Gin**	**Gin**
		Z1	**Z2**	**Z3**	**Z4**	**Z5**
Colour	**Yellow green**	4	4	-	-	-
	Yellow-straw	7	5	-	-	-
	Golden	-	-	3	-	-
	Topaz	-	-	2	-	-
	Colourless	-	-	-	10	10
Appearance	**Clear**	9	6	2	10	7
	Brilliant	3	-	-	-	-
	Particles in suspension	-	6	6	-	-

Table VI

Olfactory attributes generated by the tasters to describe the colour and appearance of the samples. The numbers correspond to the absolute frequency of the attribute for each evaluated sample.

Atributos olfativos gerados pelos provadores para descrever a cor e a aparência das amostras. Os números correspondem à frequência absoluta do atributo para cada amostra analisada.

	Portuguese juniper flavoured drinks (commercial samples)			London Gin	Gin
	Z1	Z2	Z3	Z4	Z5
Alcohol	6	5	2	2	2
Floral	7	-	-	4	5
Lavender	2	-	-	2	-
Geranium	2	-	-	-	-
Fresh	2	-	-	-	-
Eucalyptus	2	-	-	-	-
Menthol	2	-	-	-	-
Resin	-	-	-	-	4
Fruity	2	3	-	-	2
Vanilla	-	3	-	-	-
Caramel	-	4	2	-	-
Dry fruits	-	3	-	-	-
Walnut	-	2	3	-	-
Almond	-	-	2	-	-
Honey	-	2	5	-	-
Sweet	-	3	5	-	-
Dried fruits	-	-	2	-	-
Woody	-	-	4	-	-
Vegetal/herbaceous	-	-	2	-	2
Marc	-	5	-	-	-
Citrus	-	-	-	-	4
Rose	-	-	-	2	-
Gin	-	-	-	2	-
Spicy	-	-	-	2	-

Table VII

Flavour attributes generated by the tasters to describe the colour and appearance of the samples. The numbers correspond to the absolute frequency of the attribute for each evaluated sample.

Atributos de sabor gerados pelos provadores para descrever a cor e a aparência das amostras. Os números correspondem à frequência absoluta do atributo para cada amostra analisada.

	Portuguese juniper flavoured drinks (commercial samples)			London Gin	Gin
	Z_1	Z_2	Z_3	Z_4	Z_5
Alcoholic/alcohol	5	5	3	5	3
Burning/warm	2	3	-	3	0
Sweet	0	3	2	2	2
Smooth	2	4	2	3	3
Bitterness	-	-	3	-	2
Sour	-	-	2	-	2
Floral	-	-	-	3	-
Menthol	2	-	-	2	-
Citrus	-	-	-	2	-
Vegetal/herbaceous	-	-	-	-	3
Fruity	2	-	-	-	-
Vanilla	-	2	-	-	-
Marc	-	5	-	-	-
Persistent	-	2	2	-	-
Harshness	-	2	2	-	-
Walnut	-	-	2	-	-
Complexity	-	-	2	-	-

also been used to describe the gin flavour (Piggott and Holm, 1983; Riu-Aumatell *et al.*, 2008).

The drinks seem to have a different sensory profile with the exception for the alcohol attribute assigned in all of them. This is a common descriptor of other spirits such as spirits aged in wood (Caldeira *et al.*, 2006). Even the Portuguese flavoured juniper drinks present different profiles, which is in accordance with the physicochemical analysis.

In sample Z2 marc attribute was detected with high frequency, which seems to suggest the use of grape marc spirit together with arbutus spirit, in the preparation of the sample Z2, which is in accordance with chemical analysis. The vanilla attribute in sample Z2 must be due to the addition of vanilla according to the label information. This sample is also described by caramel, dry fruits, sweet, walnut and honey.

The sensory profile of the Z3 sample is fairly similar to the Z2 sample, but from what is known about this drink, no substance has been added, so the presence of these descriptors can result from aging in wood, just as stated in wooden aged wine brandies (Belchior *et al.*, 2001; Caldeira *et al.*, 2006; Caldeira *et al.*, 2008; Caldeira *et al.*, 2010). In this sample the wood attribute was also found, which distinguishes it from the other beverages. Samples Z4 and Z5 have similar aromas, including floral, citrus and alcohol which are well associated with the gin flavour (McDonnell *et al.*, 2001). In terms of flavour, Z1 and Z4 are very similar in the attributes of alcohol, burning, smooth and menthol.

The tasters ranked the similarity/dissimilarity of different pairs of drinks based on a scale of 1 to 5, where 1 corresponds to very different and 5 very similar. Figure 1 shows the average values assigned by the

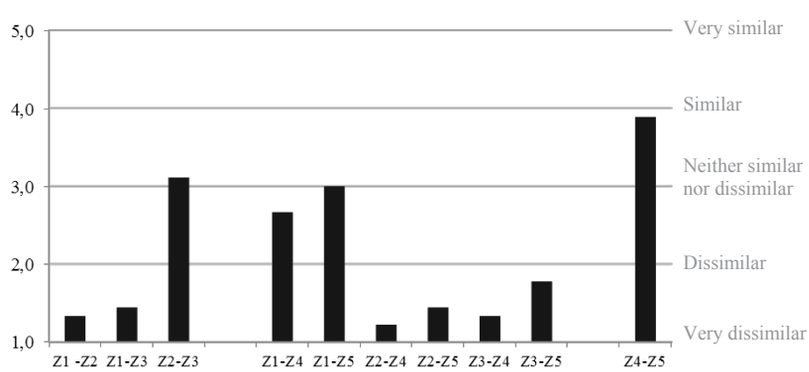

Figure 1 – Average value of the tasting panel in the similarity/dissimilarity (1-very dissimilar; 5-very similar) to the different pairs of drinks.

Valor médio do painel de prova na semelhança/diferença (1-muito diferente; 5-muito semelhante) para os diferentes pares de bebidas.

panel of tasters to each different pair of drink. This additional information intends to check the sensory differences of these beverages.

The pairs of drinks with a high similarity are samples Z4 - Z5 and Z2 - Z3. In contrast the Z1 sample is very different from the Z2 and Z3 samples in the group of Portuguese juniper flavoured drinks, while it is closer to the London gin (Z4) and gin sample (Z5). These results are in accordance with physicochemical analysis.

CONCLUSIONS

The first evaluation of commercial and non-commercial samples of the Portuguese drink called "aguardente de zimbro", which is made by the maceration of juniper berries with several distillates, put in evidence several physicochemical and sensory profiles on the analysed samples. Thus, further samples must be analysed in order to understand the existence or not of a characteristic profile associated with this type of alcoholic drink. In addition further research is needed in order to study the effects of the technological variables like the spirit used, quantity of junipers berries and maceration time on the composition of the drink.

With respect to the three commercial products found at the market, it seems that the producers are innovating by using vanilla, wood ageing or using a different distillate with influence on the sensory profile of the drinks, in spite of a yellow colour for all of them.

Nevertheless, this work presents a first approach to the sensory and physicochemical characterisation of this flavoured juniper drink that could promote future research.

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