

First Approach to the Analytical Characterization of Barrel-Aged Grape Marc Distillates Using Phenolic Compounds and Colour Parameters

Raquel Rodríguez-Solana^{1,2}, José Manuel Salgado³, José Manuel Domínguez^{1,2} and Sandra Cortés-Diéguez^{1,2*}

¹Department of Chemical Engineering, Sciences Faculty, University of Vigo, Campus Ourense, As Lagoas s/n, ES-32004 Ourense, Spain

²Laboratory of Agro-food Biotechnology, CITI-Tecnópole, Parque Tecnológico de Galicia, San Cibrao das Viñas, ES-32900 Ourense, Spain

³IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, PT-4710-057 Braga, Portugal

Received: February 27, 2014

Accepted: October 9, 2014

Summary

Phenolic compounds (benzoic and cinnamic acid derivatives) were determined by high-performance liquid chromatography with multiple wavelength detector (HPLC-MWD) in grape marc distillates aged in *Quercus petraea*, *Quercus robur* and *Quercus alba* wooden barrels. In addition to colour indices and evaluable polyphenols, all samples were described by sensorial analysis. There were significant differences in the mean concentrations of the majority of phenolic compounds among the samples. Gallic and benzoic acids were the most abundant and samples aged in *Q. robur* from Galicia (NW of Spain) had the highest concentration of most of the determined phenols. Grape marc distillates aged in *Q. robur* obtained the highest values of all sensorial attributes, whereas samples aged in *Q. petraea* and *Q. alba* obtained similar scores. Principal component analysis accounted for 88.32 % of total variance, showing a good separation of aged distillates in terms of phenolic compounds and colour characteristics, according to the species and origin of the oak wood used in the ageing process.

Key words: ageing, grape marc distillate, HPLC-MWD, phenols, sensory analysis, wooden barrel

Introduction

Different high-alcohol drinks can be obtained after distilling some previously fermented raw material. The most popular alcoholic beverages obtained from grape marc are orujo (Spain), bagaçoira (Portugal), grappa (Italy) and tsipouro (Greece). In the majority of these cases, freshly distilled beverages are defined with sensory attributes like sharp, alcoholic, rude and bitter, therefore ageing in oak barrels is essential to give them the sensory characteristics that consumers like (1,2).

During the maturation process, several physicochemical reactions occur in distillates, such as extraction of wood components, loss of low-boiling-point compounds from the immatured distillate by evaporation, and interactions among components from wood and beverage. As a result of different reactions such as polymerization, esterification, acetalisation, hydrolysis and oxidation (3,4), the initial product modifies its chemical composition and the sensory characteristics, visual aspect (colour and limpidity), taste and flavour (5). Low molecular mass phenolic

*Corresponding author: Phone: +34 988 387 416; Fax: +34 988 387 401; E-mail: smcortes@uvigo.es

compounds can be pointed out among the released components from wood during ageing, since they are not present in fresh distillates (6). The identification and quantification of phenols are crucial due to their influence on the chemical composition, sensory characteristics of the resulting aged distillate and also on their antioxidant activity (7,8).

The extension of these physicochemical changes depends on several factors, such as the botanical species and geographical origin of the wood (region, type of forest, climate, soil type, *etc.*), the technology of the barrel-making process (mainly the toasting intensity), the size of the barrel, their previous uses, ageing time, ageing conditions (cellar temperature and humidity) and the pH, alcohol content and total acidity of the initial wine or distillate (9–15).

Oak (genus *Quercus*) is the most suitable botanical species of wood for alcoholic drink maturation (16). *Quercus alba* (American oak), which grows in different areas in the United States, *Quercus petraea* (sessile oak) and *Quercus robur* (pedunculate oak) from Europe, are the most common oak species used in barrel-making (17). French oak is the most popular wood for the maturation of beverages around the world (18), whereas Spanish oaks, mainly grown in the North of Spain, are used at a lower scale for ageing of wines and a few studies about their behaviour have been published (19–21).

Some distilleries in Galicia (NW of Spain) also use oak from *Q. robur* grown in this area to age the grape marc distillate (called orujo). However, only a small part of the production of orujo is subjected to an ageing process, due to significant expenses: wooden barrels are expensive, and this cost increases in proportion to the length of the ageing period, mainly due to alcohol evaporation.

The physicochemical parameters of these beverages are fixed by the Regulatory Council in the control program (22). These parameters are the same as in young orujo and only change the minimum and maximum values fixed for them, although the ageing process changes the chemical composition of young distillate and as consequence its sensory profile (23). The influence of the species of oak wood and time of ageing on the mineral composition of distillate was also observed (24), and the results

showed a great influence of the species of oak wood on the final composition of the distillate.

The current study has been undertaken to obtain the descriptive phenolic and sensory attributes of aged orujo distillates, in order to characterize these alcoholic beverages for the first time. The results can also be useful to value the suitability of employing oak barrels grown in Spain as an alternative to the barrels bought from other countries and thus contributing to the improvement of the quality of these distillates and the attempt to reduce the high initial cost.

Materials and Methods

Samples

Ten commercial grape marc distillates from Galicia (orujo) aged in 225-litre wooden barrels from the species *Quercus robur* (pedunculate oak; origin: Limousin, France and Galicia, Spain), *Quercus alba* (American oak) and *Quercus petraea* (origin: Allier, France) were analysed. These samples correspond to all the types of barrels currently present on the market. They were collected and bottled in glass bottles after an ageing period between 1 and 6 years. General information about aged orujo (species of oak wood, time of ageing, *etc.*) was supplied by the producers. In Table 1 all the information concerning the analysed samples is summarized.

Reagents

The chemical standards used: gallic acid (3,4,5-trihydroxybenzoic acid) monohydrate, sinapic acid, syringaldehyde (3,5-dimethoxy-4-hydroxybenzaldehyde), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) and vanillin were purchased from Fluka (Madrid, Spain). Benzoic acid, coniferaldehyde (4'-hydroxy-3'-methoxycinnamaldehyde), ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid), isoferulic acid (3-hydroxy-4-methoxycinnamic acid), *p*-coumaric acid (4-hydroxycinnamic acid), sinapaldehyde (*trans*-3,5-dimethoxy-4-hydroxycinnamaldehyde), and 4-hydroxybenzaldehyde were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Protocatechualdehyde (3,4-dihydroxybenzaldehyde), vanillyl alcohol (4-hydroxy-

Table 1. Main characteristics of the analysed orujo distillates aged in different oak wood barrels

Oak species	Sample code	Grape variety	Producer code*	Distillation equipment	Geographical location	φ (alcohol) %	t (ageing) month
<i>Quercus robur</i>	QRL13	Mencia	A	steam	Limousin (France)	38.7	13
	QRL16	Albariño	B	steam	Limousin (France)	40.5	16
	QRG60	Albariño	C	alembic	Galicia (Spain)	49.0	60
	QRG72	Albariño	C	alembic	Galicia (Spain)	44.8	72
<i>Quercus petraea</i>	QPA60	Albariño	D	steam	Allier (France)	47.6	60
	QPA72	mix of red grape varieties	A	alembic	Allier (France)	50.0	72
	QPA72B	Albariño	F	steam	Allier (France)	40.3	72
	QPA144	Godello	G	steam	Allier (France)	43.4	144
<i>Quercus alba</i>	QA72A	Albariño	F	steam	USA	44.8	72
	QA72B	Albariño	F	steam	USA	46.1	72

*Different letters represent different companies

-3-methoxybenzyl alcohol), and vanillic acid (4-hydroxy-3-methoxybenzoic acid) were purchased from SAFC Sigma-Aldrich. Absolute ethanol was purchased from Merck (Darmstadt, Germany). Methanol (HPLC-gradient grade) and formic acid were from Panreac (Barcelona, Spain), and Milli-Q water from Millipore system (Bedford, MA, USA).

Total phenolic content

According to Spigno *et al.* (25), total phenols were determined applying two methods: (i) Singleton and Rossi procedure (26) using Folin-Ciocalteu reagent, the best method to determine total phenols, including tanins, and (ii) direct measure of the absorbance of each sample at 280 nm ($A_{280\text{ nm}}$) employing a one-centimetre quartz cuvette (27). All samples were previously diluted 20 or 100 times. Total phenolic index (TPI) was determined by the following equation:

$$\text{TPI} = A_{280\text{ nm}} \cdot \text{dilution factor} \quad /1/$$

The quantification of both parameters was carried out using a calibration curve with known concentrations of gallic acid and expressed as gallic acid equivalents (GAE).

Colour intensity and hue

Colour intensity and hue, determined by absorbance measurements of diluted or undiluted samples at the wavelengths of 420 (yellow), 520 (red) and 620 nm (violet), were also evaluated employing one-centimetre polystyrene cuvettes (28,29). The equations for the calculation of colour intensity (CI) and hue (h) were as follows:

$$\text{CI} = A_{420\text{ nm}} + A_{520\text{ nm}} + A_{620\text{ nm}} \quad /2/$$

and

$$h = A_{420\text{ nm}} / A_{520\text{ nm}} \quad /3/$$

Spectrophotometric analyses were performed using a UV-VIS Cintra 6 spectrophotometer (GBC Scientific Equipment, Madrid, Spain).

HPLC-MWD analysis

All samples of aged grape marc spirits were filtered through 0.45- μm pore membranes of cellulose acetate (Sartorius, Goettingen, Germany) before the analysis using high-performance liquid chromatography (HPLC). An Agilent Technologies 1200 series system consisted of a quaternary pump (G1311A), an injector, a degasser (G1322A), a multiple wavelength detector (MWD, UV/VIS; Agilent, Palo Alto, CA, USA) and a Zorbax SB-Aq reversed-phase column, 5 μm , 150 mm \times 4.6 mm i.d. (Agilent) with a guard column. Samples of 20 μL of aged spirit or calibration standards were injected into the column and eluted with the following gradient: solvent A (methanol) and solvent B (2.5 % formic acid in Milli-Q water, by volume) at a flow rate of 1 mL/min. Zero time conditions were 100 % B, after 35 min the pumps were adjusted to 52 % B and 48 % A, at 56 min to 100 % A until the end of the analysis at 65 min. Detection was carried out at (276 \pm 4) nm. The identification of each compound was done by comparing the retention times with those of pure standards. All determinations were made in duplicate.

Sensory analysis

The panel for sensory analysis consisted of eight assessors, five males and three females aged from 35 to 55, all of them members of the official panel of Geographic Denomination of Spirits and Liqueurs from Galicia, expert tasters in sensory analysis of this kind of alcoholic beverages. The selected judges worked directly for wineries and distilleries either as winemakers or in marketing. The sensory analysis was performed in a laboratory, containing 20 independent tasting booths, and designed according to the International Organization for Standardization, standard ISO 8589 (30).

Before evaluation, during three training sessions (12 h), a collection of six representative samples was tested by the panellists in order to generate relevant appearance and taste attributes. All samples were commercially available. The aim of these sessions was to develop a common vocabulary for the description of the sensory attributes of aged orujo samples. In the first phase of this training, the judges identified thirty-three descriptors (13 for appearance and 20 for taste). After a round-table discussion and by consensus, the panel selected and refined the attributes that best describe their perceptions. Synonymous, hedonic and irrelevant descriptors were also eliminated by using statistical methods described in ISO 11035 standard (31). Finally, the generated attributes were reduced to eight (three for appearance and five for taste).

In the formal session, the intensity of each attribute was rated on a five-point scale, where 0 indicated that the descriptor was not perceived, 1=low, 2=low-medium, 3=medium, 4=medium-high and 5=high. Each sample was previously coded and presented to judges in random order. The sensory evaluation was carried out at room temperature, using the official glasses of the corresponding Regulation Commission. Tasting was carried out in the morning during five sessions on different days to avoid fatigue of the tasters due to the high degree of alcohol in the aged grape marc distillates (37.5–50 %, by volume).

Statistical analysis

The obtained results were analysed using XLSTAT-Pro (Addinsoft, Paris, France). One-way analysis of variance (ANOVA) was applied to establish whether significant differences ($p < 0.05$) existed between the values obtained for the mean concentration of each compound in the analysed aged grape marc distillates. The multiple range test (LSD) was applied to confirm the obtained results. Pearson's correlations among all identified phenolic compounds, between sensorial descriptors and phenolic compounds, and between sensorial attributes and colour parameters were also calculated. In order to determine the influence of the oak species on the composition of grape marc distillates, a multivariate principal component analysis was carried out.

Results and Discussion

Spectrophotometric parameters of aged orujo distillates

Table 2 lists all phenolic indices and chromatic characteristics of the analysed samples. Taking into account only

Table 2. Concentration of total phenols and chromatic characteristics of orujo distillates after ageing in barrels

Oak species	<i>Quercus robur</i>		<i>Quercus petraea</i>	<i>Quercus alba</i>
Geographical location	Limousin	Galicia	Allier	USA
FCI/(mg of GAE per L)	626±12	451±33	482±63	402±303
TPI/(mg of GAE per L)	(1232±1052) ^a	(5590±352) ^b	(1728±1051) ^a	(1211±44) ^a
CI	(0.9±0.5) ^{ac}	(2.3±0.3) ^b	(1.5±0.2) ^a	(0.7±0.2) ^c
<i>h</i>	(4.8±0.3) ^a	(5.9±0.3) ^b	(5.3±0.5) ^{ab}	(5.4±0.1) ^{ab}

*Different letters in superscript within the same row indicate statistically significant differences ($p \leq 0.05$) according to LSD test
FCI=Folin-Ciocalteu index, TPI=total phenolic index, GAE=gallic acid equivalent, CI=colour intensity, *h*=hue

the species of oak, no significant differences were observed in the Folin-Ciocalteu index among the samples; however, the values of other spectrophotometric parameters were significantly different. Higher contents of total phenols were observed in the group of distillates aged in *Q. robur* (Galicia), showing significantly different value with respect to the other analysed distillates, including those aged in the same oak species of other geographical origin. Total phenolic index (TPI) determined in each group of distillates showed higher values than the corresponding total phenols evaluated with Folin-Ciocalteu method.

Orujo aged in *Q. robur* (Galicia) showed the highest values of colour intensity, whereas the samples aged in *Q. alba* showed the lowest. Hue value was lower in the samples aged in *Q. robur* (Limousin); however, other samples did not show significant differences. The results showed a great influence of the wood origin on the chromatic characteristics of the samples, since significant differences were found among distillates aged in *Quercus robur* from different areas.

Validation of the HPLC method

The validation of the method was done based on linearity and analytical limits (limits of detection and quantification).

Linearity

The study of the linearity was performed using the HPLC analysis of seven standard solutions containing increasing concentrations of their respective standards covering the range of linearity. These solutions were prepared in triplicate in ultrapure water with 40 % (by volume) of absolute ethanol and filtered through 0.45- μ m pore membranes of cellulose acetate. Data in Table 2 show that linearity is satisfactory in almost all cases, with the correlation coefficient (R^2) ranging from 0.9712 (4-hydroxybenzaldehyde) to 0.9999 (coniferaldehyde).

Analytical limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the parameters of the analytical curves. Both limits were calculated according to the following mathematical relationships:

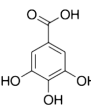
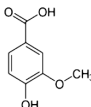
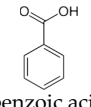
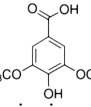
$$\text{LOD} = 3 \cdot \text{S.D.} / m \quad /4/$$

and

$$\text{LOQ} = 10 \cdot \text{S.D.} / m \quad /5/$$

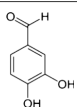
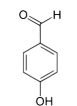
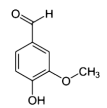
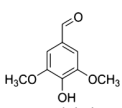
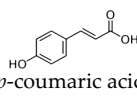
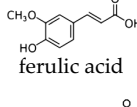
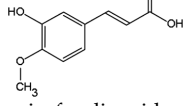
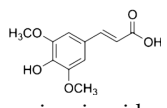
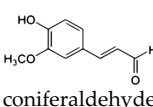
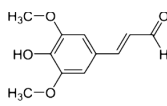
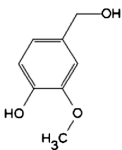
where S.D. is the estimation of the standard deviation of the regression line, and *m* is the slope of the calibration curve (32). The LOD and LOQ were low for all determined phenolic compounds (Table 3).

Table 3. Family, compound, retention time, linearity parameters and analytical limits of the HPLC method for the quantification of phenolic compounds in aged grape marc distillates

Family	Compound	t_R /min	a	S.D. ^a	b	S.D. ^b	R^2	LOD	LOQ
	 gallic acid	5.1	60.30	32.52	17.08	0.89	0.9987	2.93	9.78
Benzoic acids	 vanillic acid	17.59	39.04	3.74	4.83	0.85	0.9972	0.48	1.61
	 benzoic acid	20.02	0.94	2.72	1.83	0.10	0.9994	0.39	1.31
	 syringic acid	21.75	58.76	1.7	1.11	0.36	0.9997	0.17	0.57

t_R =retention time (min), a=slope, S.D.^a=standard deviation of the slope, b=intercept, S.D.^b=standard deviation of the intercept, R^2 =correlation coefficient of linear range, LOD=limit of detection, LOQ=limit of quantification

Table 3. – continued

Family	Compound	t_R /min	a	S.D. ^a	b	S.D. ^b	R ²	LOD	LOQ
Benzoic aldehydes	 protocatechualdehyde	10.91	417.64	6.26	-2.63	30.33	0.9846	0.05	0.17
	 4-hydroxybenzaldehyde	15.03	513.06	10.36	2.44	39.01	0.9712	0.07	0.22
	 vanillin	20.62	72.98	4.07	4.54	0.59	0.9996	0.27	0.92
	 syringaldehyde	24.36	28.83	4.02	4.86	0.32	0.9994	0.59	1.97
Cinnamic acids	 <i>p</i> -coumaric acid	22.82	74.09	3.59	-7.82	2.77	0.993	0.51	1.7
	 ferulic acid	25.9	227.34	11.43	-99.42	6.15	0.9998	0.19	0.64
	 isoferulic acid	27	60.01	1.1	-1.02	1.1	0.9983	0.07	0.25
	 sinapic acid	28.26	20.88	6.19	-4.87	0.64	0.9954	1.22	4.06
Cinnamic aldehydes	 coniferaldehyde	27.49	13.52	0.65	1.24	0.04	0.9999	0.21	0.68
	 sinapaldehyde	29.71	24.06	1.23	-0.40	0.19	0.9998	0.16	0.55
Benzoic alcohol	 vanillyl alcohol	9.98	17.77	0.94	2.60	0.38	0.9935	0.23	0.76

t_R =retention time (min), a=slope, S.D.^a=standard deviation of the slope, b=intercept, S.D.^b=standard deviation of the intercept, R²= correlation coefficient of linear range, LOD=limit of detection, LOQ=limit of quantification

Concentration of phenolic compounds in grape marc distillates aged in different wooden barrels

Fig. 1 shows the molecular structures of the compounds extracted from the wood. The phenolic aldehydes

are produced by thermodegradation of the terminal monomer units of lignin: the cinnamic aldehydes convert to benzoic aldehydes, and then they are oxidized to phenolic acids (19). Table 4 reports the concentrations of the

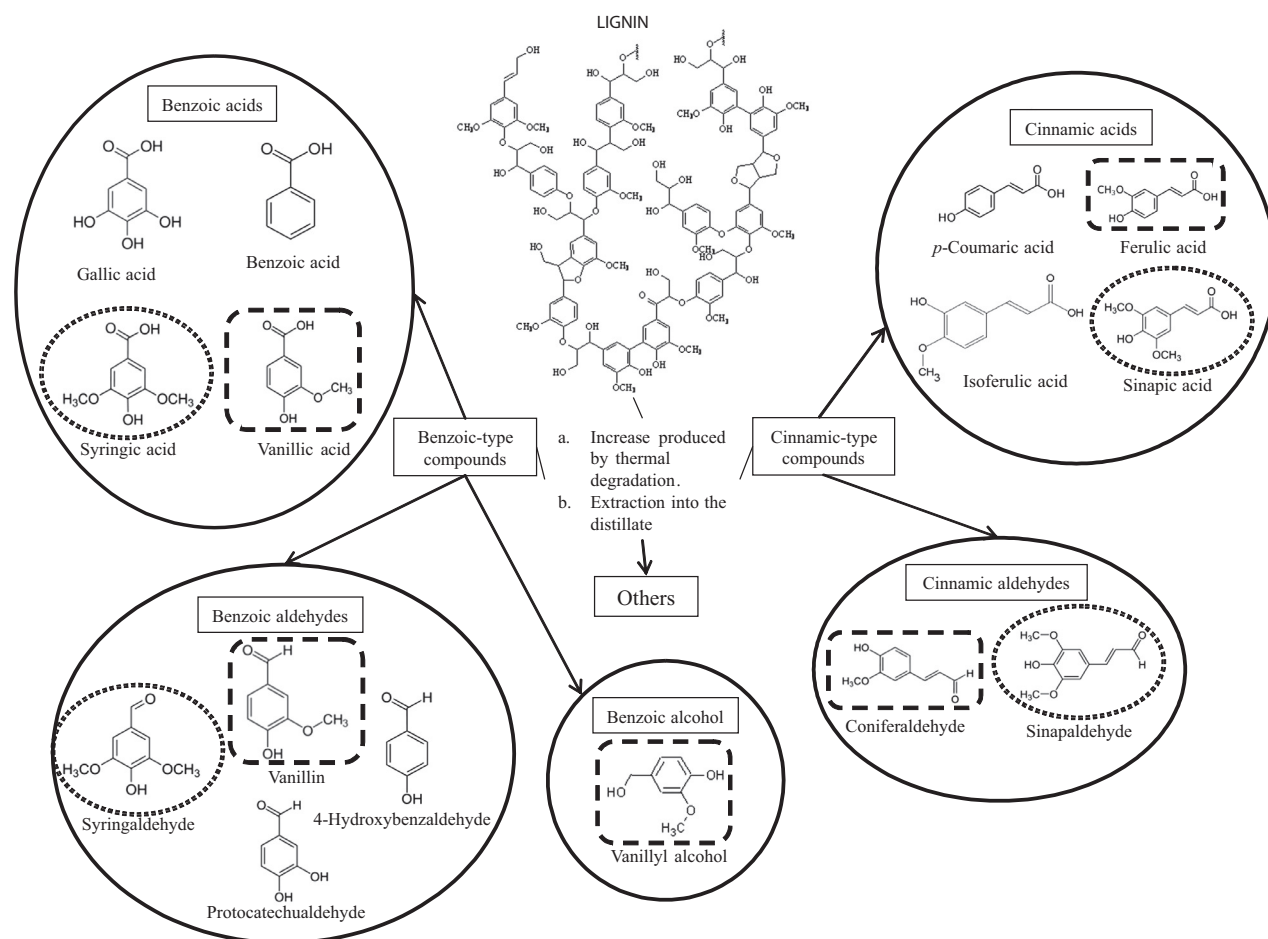


Fig. 1. Molecular structures of the phenolic compounds extracted to the grape marc distillates after ageing in oak barrels. The structures circled with dashed line in bold are guaiacyl compounds, while the structures circled with double dashed line are syringyl-type compounds

quantified phenolic compounds in the analysed aged distillates. The concentration of gallic acid in aged beverages depends on the toast level of barrels, since gallic acid is degraded at high temperatures (33). Consequently, this compound is more abundant in distillates aged in barrels with light or medium toast levels (7). Besides gallic acid, the concentration of ferulic and vanillic acids also decreased with higher temperature during the toasting process (34). The content of gallic acid was significantly higher in distillates aged in *Quercus robur* from Galicia (>50 mg/L). This compound was also present, but at low concentration, in distillates aged in *Quercus petraea* from Allier. Vanillic acid can be directly extracted from oak wood or be formed by oxidation of vanillin during the ageing process, whereas syringic acid is formed during toasting by the oxidation of the corresponding aldehyde. Both compounds were present at higher concentrations in distillates aged in *Quercus robur* from Galicia. In contrast, distillates in the same species from Limousin showed lower values of these compounds.

Vanillin and syringaldehyde are phenolic compounds related to lignin. Vanillin was detected in all analysed samples, with significantly lower concentration in the samples aged in *Quercus robur* from Limousin and higher in distillates aged in the same species from Galicia. Vanil-

lin is the phenolic aldehyde which greatly influences the aroma of distillates because of its low threshold value (320 µg/L) and adds positive vanilla notes (35). In the analysed samples, syringaldehyde was the most abundant phenolic aldehyde.

Thermal degradation of lignin leads to the formation of some phenolic alcohols (19). The concentration of vanillyl alcohol was higher in the spirit aged in *Quercus robur* (Galicia), whereas this compound was not present in distillates aged in *Quercus robur* (Limousin) or *Quercus alba*.

Ferulic acid was used in this study as a discriminating compound of the oak wood species because it can only be quantified in *Quercus robur* (Galicia). Similar results were obtained by Canas *et al.* (7), showing that the brandies aged in Portuguese oak (*Quercus pyrenaica* Willd.) contain higher values of this compound than other brandies aged in other oak species. The geographical proximity and similar climatic conditions of both areas (Galicia and northern Portugal) may be the main reasons to explain these similarities, despite the fact that the spirits aged in two different species of *Quercus*.

The presence and concentration of benzoic and cinnamic aldehydes, which arise from lignin degradation, depend on the temperature applied during toasting (16,36,37). Results in this study showed that coniferalde-

Table 4. Concentration of phenolic compounds in ten grape marc distillates aged in three different oak species during different periods of time (ANOVA results are also shown)

		γ /(mg/mL)			
Oak species		<i>Quercus robur</i>		<i>Quercus petraea</i>	<i>Quercus alba</i>
Geographical location		Limousin	Galicia	Allier	USA
Samples		2	2	4	2
Benzoic acids	gallic acid	<LOQ	(58.2±9.2) ^a	(15.6±1.3) ^b	<LOQ
	vanillic acid	<LOQ	(2.8±1.5) ^a	(2.4±0.7) ^a	(2.1±0.0) ^a
	benzoic acid	(12.2±13.0) ^a	(30.1±10.9) ^a	(14.3±4.5) ^a	(15.7±4.1) ^a
	syringic acid	(1.2±0.1) ^a	(5.8±0.7) ^b	(3.6±1.2) ^c	(3.1±0.9) ^c
Benzoic aldehydes	protocatechualdehyde	<LOQ	<LOQ	<LOQ	<LOQ
	4-hydroxybenzaldehyde	<LOQ	(0.3±0.0) ^a	(0.4±0.2) ^a	<LOQ
	vanillin	(1.3±0.4) ^a	(5.7±1.6) ^b	(3.8±1.2) ^b	(3.8±1.1) ^{ab}
	syringaldehyde	(3.2±1.2) ^a	(12.2±3.7) ^b	(8.2±2.2) ^b	(8.2±2.4) ^b
Cinnamic acids	<i>p</i> -coumaric acid	<LOQ	<LOQ	<LOQ	<LOD
	ferulic acid	<LOQ	0.9±0.0	<LOQ	<LOQ
	isoferulic acid	(0.3±0.0) ^a	(0.8±0.0) ^b	(0.4±0.1) ^a	(0.3±0.0) ^a
	sinapic acid	<LOQ	(12.6±0.3) ^a	(5.4±0.4) ^b	<LOQ
Cinnamic aldehydes	coniferaldehyde	(1.4±0.9) ^a	(13.5±0.7) ^b	(9.1±7.5) ^b	(2.0±0.4) ^a
	sinapaldehyde	(3.6±1.4) ^a	(7.7±0.7) ^b	(6.3±2.6) ^{ab}	(4.2±1.2) ^{ab}
Benzoic alcohol	vanillyl alcohol	<LOQ	(2.5±0.4) ^a	(0.9±0.1) ^b	<LOD
Ratio 1	gallic acid/vanillin	–	10.2	4.1	–
Ratio 2	syringaldehyde/vanillin	2.5	2.1	2.1	2.1

*Different letters in superscripts within the same row indicate statistically significant differences ($p \leq 0.05$) according to LSD test
LOQ=limit of quantification, LOD=limit of detection

hyde and sinapaldehyde were present at low concentrations in samples from *Quercus robur* (Limousin) and *Quercus alba*. In all cases, their individual values were higher than the corresponding benzoic aldehydes, 4-hydroxybenzaldehyde and vanillin.

Fig. 2 shows the differences between the four groups of grape marc distillates according to the total concentration of phenols from each family. Most of the identified phenolic compounds were at higher concentrations in the orujo samples aged in *Quercus robur* (Galicia) than in the samples aged in the same species of *Quercus* from Limousin. Between the other two species, orujo distillates aged in *Quercus alba* had lower concentrations of most of the

determined phenolic compounds than the distillates aged in *Quercus petraea*.

A significant effect of oak wood species was observed on 11 of the 15 studied compounds (Table 4). Benzoic acid, gallic acid and syringaldehyde were the main low molecular mass compounds in the analysed samples. The results are in agreement with those previously obtained by dos Anjos *et al.* (32) in a study about cachaça. The concentration of minor compounds such as vanillin, syringic and ferulic acids was significantly higher in distillates aged in *Quercus robur* from Galicia. Ferulic acid was only quantified in these samples. Distillates aged in *Quercus robur* from Limousin had the lowest concentration of all determined phenolic compounds. In the majority of cases, their concentrations were lower than their corresponding detection and quantification limits.

Quality and authenticity of aged grape marc distillates determined by phenol ratios

Van Jaarsveld *et al.* (18) and Gimenez-Martínez *et al.* (38) showed that the gallic acid/vanillin ratio is influenced by the type of wood and it has been used to define the quality of spirits. A higher gallic acid/vanillin ratio indicates medium to high quality brandy (39). According to van Jaarsveld *et al.* (40), the gallic acid/vanillin ratio increases with the level of toasting. In this study (Table 4) this relationship was significantly higher in distillates

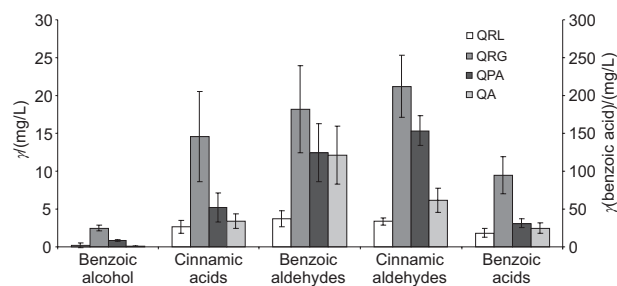


Fig. 2. Mean and standard deviation of phenolic compound families in the different species of *Quercus*. QRL=*Quercus robur* (Limousin), QRG=*Quercus robur* (Galicia), QPA=*Quercus petraea* (Allier), QA=*Quercus alba* (USA)

aged in *Quercus robur* from Galicia (10.2) than in distillates aged in *Quercus petraea* from Allier (4.1).

Other authors (39,41) also used the syringaldehyde/vanillin ratio to evaluate the quality of aged beverages. Usually the syringaldehyde content exceeds more than twice the vanillin content in oak wood species (42). In this study, the syringaldehyde/vanillin ratio values were in all cases near 2. A relationship between syringaldehyde and vanillin in the range from 1.4 to 2.5 shows a balanced lignin composition (38). No differences were found in this ratio among the species of oak wood. The syringaldehyde/vanillin ratio may also be used to evaluate the possible addition of commercial vanillin as flavouring to increase the aroma of aged distillate. In addition, the relationship between benzoic aldehydes (vanillin and syringaldehyde) and cinnamic aldehydes (coniferaldehyde and sinapaldehyde) can be used to evaluate the authenticity of the aged distillates. Canas *et al.* (37) showed that the relationship between both aldehyde families allows differentiating the type of wood (chestnut or oak) used in the ageing process. In this study, the relationship between both groups of aldehydes is shown in Fig. 3. Orujo aged in *Quercus alba* had the highest ratio of benzoic/cinnamic aldehydes (1.95), whereas samples aged in *Quercus petraea* had lower ratio than the unity (0.79). Similar values were observed in the samples of orujo aged in *Quercus robur* from Galicia and from Limousin, with a ratio of 1.07 and 0.84, respectively.

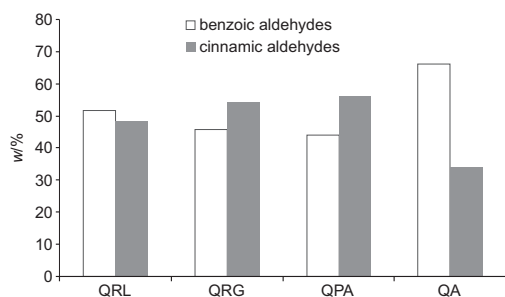


Fig. 3. Proportion of benzoic and cinnamic aldehydes as a function of the botanical species. QRL=*Quercus robur* (Limousine), QRG=*Quercus robur* (Galicia), QPA=*Quercus petraea* (Allier), QA=*Quercus alba* (USA)

Sensory analysis of aged grape marc distillates

Eight common descriptors (three for appearance and five for taste) were defined by the judges to describe the samples. The mean intensity of each attribute was used to define the sensory profile of the evaluated orujo samples (Fig. 4). The obtained results showed that the orujo samples aged in *Quercus robur* from Galicia reached the highest values of all descriptive parameters, which were close to the values for samples aged in *Quercus robur* from Limousin. Samples aged in *Quercus alba* had lower colour intensity and lower scores of most of the evaluated attributes. Orujo samples aged in *Quercus petraea* and *Quercus alba* showed similar values in positive taste parameters (sweet, dense and oily), but the ageing process in *Quercus petraea* increased some negative notes, giving these distillates more spicy/pungent and alcoholic taste. Samples aged in *Quercus petraea* had the lowest visual score, having poor brightness and transparency.

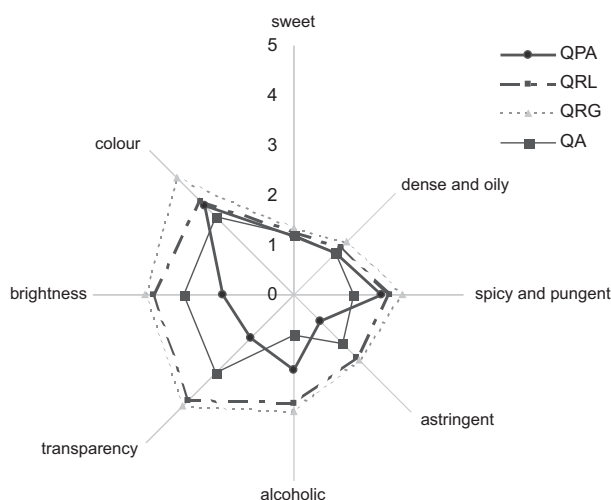


Fig. 4. Mean sensory profile of the appearance and taste of aged grape marc distillates. QRL=*Quercus robur* (Limousin), QRG=*Quercus robur* (Galicia), QPA=*Quercus petraea* (Allier), QA=*Quercus alba* (USA)

Pearson correlation coefficients

Pearson correlation coefficients among all identified phenolic compounds are shown in Table 5. The majority of the compounds were positively correlated, with values of R above 0.6. Vanillin and syringaldehyde are compounds related to lignin, but no correlation was established between them. However, vanillic and syringic acids, both products of lignin degradation, showed a high positive correlation (0.714). Both benzoic acids were also positively correlated with guaiacyl-type compounds (syringaldehyde and sinapaldehyde). Gallic acid showed high positive correlation with the majority of phenolic and cinnamic acids and with the corresponding aldehydes. Cinnamic acids (ferulic, isoferulic, *p*-coumaric and sinapic acids) were also highly correlated.

Pearson correlations between phenolic compounds and taste attributes of aged grape marc distillates were also evaluated (Table 6). Strong positive correlations were found between, benzoic (gallic, syringic and benzoic) and cinnamic (ferulic, isoferulic and sinapic) acids with negative descriptors of mouthfeel (astringent and alcoholic notes). On the other hand, positive taste attributes, sweet, dense and oily, showed strong positive correlations with the corresponding benzoic (protocatechualdehyde and syringaldehyde) and cinnamic (sinapaldehyde) aldehydes. The 4-hydroxybenzaldehyde and *p*-coumaric acid were negatively correlated with the positive attributes, whereas vanillin, as alcohol, aldehyde and acid showed strong positive correlation with the alcoholic note.

Colour parameters and the three visual attributes were also correlated (Table 7). The results showed that hue is the colour parameter that has the most influence on the positive valorisation of the aged distillates.

Principal component analysis

Fig. 5 shows the score plot of the first two PCs, obtained with the individual phenols and chromatic characteristics as variables, which explain 88.32 % of the variability among the samples. Fig. 5a shows that the first principal

Table 5. Pearson correlation matrix (r) among phenolic compounds

	Vanillic acid	Syringic acid	Vanillyl alcohol	Benzoic acid	<i>p</i> -Coumaric acid	Ferulic acid	Isoferulic acid	Sinapic acid	Protocatechu-aldehyde	Vanillin	Syringaldehyde	Sinapaldehyde	4-Hydroxybenzaldehyde	Coniferaldehyde
Gallic acid	0.523	0.714	-0.139	0.929	0.587	0.969	0.923	0.977	0.584	-0.059	0.639	0.596	0.536	0.514
Vanillic acid	1.000	0.714	0.117	0.667	0.458	0.634	0.555	0.682	0.753	0.314	0.964	0.914	0.464	0.681
Syringic acid		1.000	0.132	0.764	0.644	0.697	0.787	0.779	0.841	-0.012	0.766	0.704	0.288	0.471
Vanillyl alcohol			1.000	-0.157	0.234	0.003	0.061	-0.120	0.324	-0.129	0.243	0.301	-0.140	0.049
Benzoic acid				1.000	0.447	0.918	0.920	0.965	0.721	0.201	0.718	0.729	0.694	0.685
<i>p</i> -Coumaric acid					1.000	0.588	0.659	0.578	0.344	-0.306	0.591	0.430	-0.087	0.231
Ferulic acid						1.000	0.913	0.976	0.671	-0.061	0.748	0.701	0.536	0.537
Isoferulic acid							1.000	0.925	0.704	-0.054	0.668	0.656	0.465	0.558
Sinapic acid								1.000	0.695	0.025	0.761	0.714	0.572	0.592
Protocatechu-aldehyde									1.000	0.013	0.778	0.771	0.352	0.442
Vanillin										1.000	0.216	0.423	0.754	0.751
Syringaldehyde											1.000	0.951	0.470	0.685
Sinapaldehyde												1.000	0.655	0.838
4-Hydroxybenzaldehyde													1.000	0.872
Coniferaldehyde														1.000

Correlations higher than ± 0.6 are shown in bold

Table 6. Pearson correlation matrix (r) among phenolic compounds and sensory attributes of taste

	Sweet	Dense/oily	Spicy/pungent	Astringent	Alcoholic
Gallic acid	0.098	0.256	0.363	0.726	0.841
Vanillic acid	0.183	0.487	-0.077	0.317	0.587
Syringic acid	0.100	0.358	0.190	0.591	0.869
Benzoic acid	-0.150	-0.112	0.259	0.781	0.652
Protocatechu-aldehyde	0.438	0.714	-0.162	0.479	0.316
4-Hydroxybenzaldehyde	-0.724	-0.731	-0.090	-0.334	0.300
Vanillin	0.143	0.451	-0.038	0.549	0.726
Syringaldehyde	0.622	0.820	0.346	0.243	0.428
<i>p</i> -Coumaric acid	-0.799	-0.826	-0.638	0.365	-0.023
Ferulic acid	0.119	0.303	0.274	0.782	0.819
Isoferulic acid	0.270	0.447	0.241	0.798	0.673
Sinapic acid	0.153	0.358	0.302	0.700	0.846
Coniferaldehyde	-0.500	-0.577	0.354	0.307	0.583
Sinapaldehyde	0.779	0.736	0.882	-0.227	0.147
Vanillyl alcohol	0.366	0.555	0.422	0.543	0.735

Correlations higher than ± 0.6 are shown in bold

Table 7. Pearson correlation matrix (r) among colour parameters and sensory attributes of appearance

	Transparency	Brightness	Colour
Total phenolic index	0.191	0.161	0.454
Total phenols	0.724	0.590	-0.017
CI	0.536	0.496	0.584
<i>h</i>	0.851	0.876	0.660

Correlations higher than ± 0.6 are shown in bold; CI=colour intensity, *h*=hue

component, PC1 (77.91 %), was positively correlated with all studied variables, whereas the second principal component, PC2 (10.40 %), was mainly positively correlated with the benzoic and cinnamic aldehydes and negatively with gallic and isoferulic acids and total phenols. Four groups of samples plotted on the plane defined by the two first principal components can be observed in Fig. 5b. Samples from group 1 (QRG60 and QRG72) were better characterized by all variables associated with the positive side of PC1, mainly by gallic, syringic and sinapic acids and by total phenols and colour intensity. In contrast,

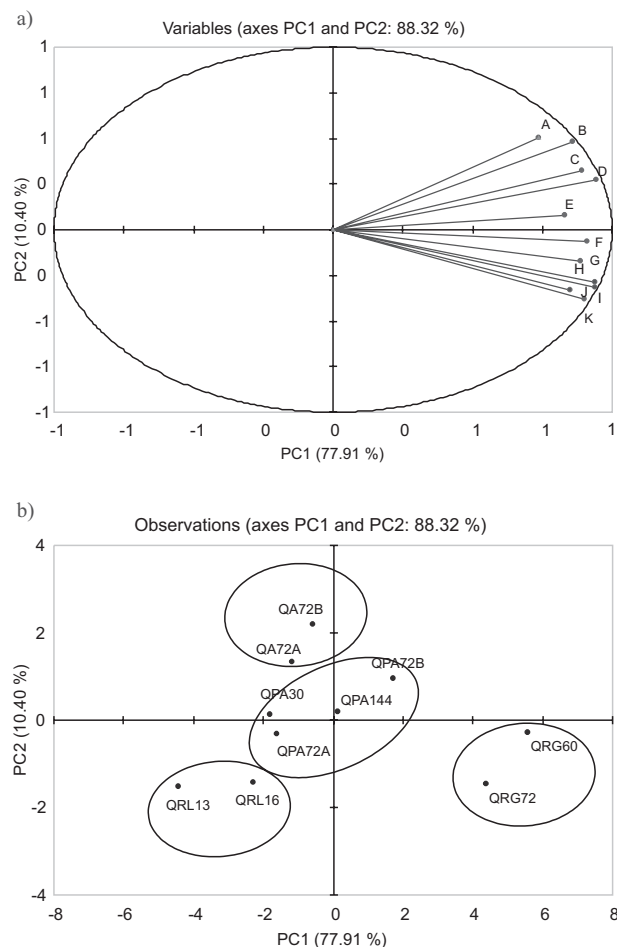


Fig. 5. Principal component analysis score plot of: a) aged grape marc distillates, and b) phenolic compound variables. QRL=*Quercus robur* (Limousin), QRG=*Quercus robur* (Galicia), QPA=*Quercus petraea* (Allier), QA=*Quercus alba* (USA). The number reflects the ageing time. A=sinapaldehyde, B=syringaldehyde, C=vanillin, D=syringic acid, E=hue, F=colour intensity, G=vanillyl alcohol, H=sinapic acid, I=gallic acid, J=total phenols, K=isoferulic acid

samples included in group 2 (QRL13 and QRL16) were scarcely characterized by these variables, showing differences among samples aged in the same species of oak wood of different origin. Group 3 (QA72A and QA72B) was characterized by volatiles on the positive side of PC2, mainly syringaldehyde and sinapaldehyde. Group 4 was composed of distillates aged in *Q. petraea* (QPA30, QPA72A, QPA72B and QPA144) in the centre of the plot. The PCA analysis clearly showed a good separation of the aged orujo samples according to the species and origin of the oak wood employed in the ageing process, independent of the time of ageing.

Conclusions

The results obtained in this study provide the first data on the phenolic composition of the aged grape marc distillate (orujo) and contribute to the knowledge about this alcoholic beverage. Benzoic acid, gallic acid and syringaldehyde were the main low molecular mass compounds. Distillates aged in *Quercus robur* from Galicia

showed the highest concentration of the majority of the determined phenolic compounds, whereas samples aged in *Quercus robur* from Limousin had the lowest corresponding values. These results showed the influence of the growth origin on the oak composition. Ferulic acid was only detected in orujo samples aged in *Quercus robur* from Galicia. Consequently, this compound can be used as a discriminant among the three oak wood species in this study. Most of the determined phenolic compounds and colour parameters were positively correlated with each other and with the sensory attributes defined by the tasters. No significant differences of the Folin-Ciocalteu indices were shown among the analysed aged orujo beverages; however, total phenols, colour intensity and hue were significantly higher in orujo aged in *Quercus robur* from Galicia. Principal component analysis allowed the classification of the aged distillate samples according to the origin and species of oak wood. However, the results shown in this study must be completed with those obtained from an experimental design using a unique distillate aged in different oak species during the same time. Other variables such as toast level and contact time (oak-distillate) must also be taken into account in deciding which type of oak is the most suitable for ageing grape marc spirits. In this research analytical characterization of orujo alcoholic beverages is presented for the first time.

Acknowledgements

We are grateful for the financial support of this work to the Spanish Ministry of Science and Innovation (project CTQ2011-28967), and partial financial support from the FEDER funds of the European Union and to the Regulatory Board of Geographical Names of the Traditional Galician Spirits and Liqueurs for providing the samples. José Manuel Salgado is grateful for postdoctoral fellowship (EX-2010-0402) of Education Ministry of Spanish Government.

References

1. M. De Rosso, A. Panighel, A. Dalla Vedova, L. Stella, R. Flamini, Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry and oak wood barrels, *J. Agric. Food Chem.* 57 (2009) 1915-1920. <http://dx.doi.org/10.1021/jf803161r>
2. K.Y.M. Lee, A. Paterson, J.R. Piggot, G.D. Richardson, Origins of flavour in whiskies and a revised flavour wheel: A review, *J. Inst. Brew.* 107 (2001) 287-313. <http://dx.doi.org/10.1002/j.2050-0416.2001.tb00099.x>
3. V. Litchev, Influence of oxidation processes on the development of the taste and flavour of wine distillates, *Am. J. Enol. Vitic.* 40 (1989) 31-35.
4. M.J. Onishi, J.F. Guymon, E.A. Crowl, Changes in some volatile constituents of brandy during aging, *Am. J. Enol. Vitic.* 28 (1977) 152-158.
5. M.C. Rodríguez-Dodero, D.A. Guillén-Sánchez, M. Schwarz-Rodríguez, C. García-Barroso, Phenolic compounds and furanic derivatives in the characterization and quality control of Brandy de Jerez, *J. Agric. Food Chem.* 58 (2010) 990-997. <http://dx.doi.org/10.1021/jf902965p>
6. S.E. Ebeler, M.B. Terrien, C.E. Butzke, Analysis of brandy aroma by solid-phase microextraction and liquid-liquid ex-

- traction, *J. Sci. Food Agric.* 80 (2000) 625–630.
[http://dx.doi.org/10.1002/\(SICI\)1097-0010\(200004\)80:5<625::AID-JSFA584>3.0.CO;2-5](http://dx.doi.org/10.1002/(SICI)1097-0010(200004)80:5<625::AID-JSFA584>3.0.CO;2-5)
7. S. Canas, V. Casanova, A.P. Belchior, Antioxidant activity and phenolic content of Portuguese wine age brandies, *J. Food Compos. Anal.* 21 (2008) 626–633.
<http://dx.doi.org/10.1016/j.jfca.2008.07.001>
 8. D.M. Goldberg, B. Hoffman, J. Yang, G.J. Soleas, Phenolic constituents, furans, and total antioxidant status of distilled spirits, *J. Agric. Food Chem.* 47 (1999) 3978–3985.
<http://dx.doi.org/10.1021/jf9811626>
 9. T. Garde-Cerdán, C. Lorenzo, J.M. Carot, M.D. Esteve, M.D. Climent, M.R. Salinas, Effect of composition, storage time, geographic origin and oak type on the accumulation of some volatile oak compounds and ethylphenols in wines, *Food Chem.* 122 (2010) 1076–1082.
<http://dx.doi.org/10.1016/j.foodchem.2010.03.077>
 10. L. Matricardi, A.L. Waterhouse, Influence of toasting technique on color and ellagitannins of oak wood in barrel making, *Am. J. Enol. Vitic.* 50 (1999) 519–526.
 11. D.P. Miller, G.S. Howell, C.S. Michaelis, D.I. Dickmann, The content of phenolic acid and aldehyde flavor components of white oak as affected by site and species, *Am. J. Enol. Vitic.* 43 (1992) 333–338.
 12. A. Prida, J.L. Puech, Influence of geographical origin and botanical species on the content of extractives in American, French, and East European oak woods, *J. Agric. Food Chem.* 54 (2006) 8115–8126.
<http://dx.doi.org/10.1021/jf0616098>
 13. P. Rodríguez-Rodríguez, E. Gómez-Plaza, Differences in the extraction of volatile compounds from oak chips in wine and model solutions, *Am. J. Enol. Vitic.* 62 (2011) 127–132.
<http://dx.doi.org/10.5344/ajev.2010.10045>
 14. P. Rodríguez-Rodríguez, E. Gómez-Plaza, Dependence of oak-related volatile compounds on the physicochemical characteristics of barrel-aged wines, *Food Technol. Biotechnol.* 50 (2012) 59–65.
 15. P.J. Spillman, M.A. Sefton, R. Gawel, The effect of oak wood source, location of seasoning and coopering on the composition of volatile compounds in oak-matured wines, *Aust. J. Grape Wine Res.* 10 (2004) 216–226.
<http://dx.doi.org/10.1111/j.1755-0238.2004.tb00025.x>
 16. S. Canas, A.P. Belchior, M.I. Spranger, R. Bruno de Sousa, HPLC method for the quantification of phenolic acids, phenolic aldehydes, coumarins and furanic derivatives in different kinds of toasted wood used for the ageing of brandies, *Anal. Methods*, 3 (2011) 186–191.
<http://dx.doi.org/10.1039/c0ay00269k>
 17. P. Chatonnet, D. Dubourdiou, Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Quercus robur*) for production of barrels used in barrel ageing wines, *Am. J. Enol. Vitic.* 49 (1998) 79–85.
 18. F.P. van Jaarsveld, S. Hattingh, P. Minnaar, Rapid induction of ageing character in brandy products. Part II. Influence of type of oak, *S. Afr. J. Enol. Vitic.* 30 (2009) 16–23.
 19. E. Cadahía, L. Muñoz, B. Fernández de Simón, M.C. García-Vallejo, Changes in low molecular weight phenolic compounds in Spanish, French and American oak woods during natural seasoning and toasting, *J. Agric. Food Chem.* 49 (2001) 1790–1798.
<http://dx.doi.org/10.1021/jf0006168>
 20. B. Fernández de Simón, E. Cadahía, J. Jalocha, Volatile compounds in Spanish red wine aged in barrels made of Spanish, French and American oak wood, *J. Agric. Food Chem.* 51 (2003) 7671–7678.
<http://dx.doi.org/10.1021/jf030287u>
 21. E. Guchu, M.C. Díaz-Maroto, I.J. Díaz-Maroto, P. Vila-La-meiro, M.S. Pérez-Coello, Influence of the species and geographical location on volatile composition of spanish oak wood (*Quercus petraea* Liebl. and *Quercus robur* L.), *J. Agric. Food Chem.* 54 (2006) 3062–3066.
<http://dx.doi.org/10.1021/jf053055z>
 22. Regulation of the Geographic Indications of Traditional Spirits and Liqueurs from Galicia, DOG No. 10 (2012) 2516–2545.
 23. R. Rodríguez-Solana, N. Rodríguez, J.M. Domínguez, S. Cortés, Characterization by chemical and sensory analysis of commercial grape marc distillate (orujo) aged in oak wood, *J. Inst. Brew.* 118 (2012) 205–212.
<http://dx.doi.org/10.1002/jib.25>
 24. R. Rodríguez-Solana, J.M. Salgado, J.M. Domínguez, S. Cortés, Assessment of minerals in aged grape marc distillates by FAAS/FAES and ICP-MS. Characterization and safety evaluation, *Food Control*, 35 (2014) 49–55.
<http://dx.doi.org/10.1016/j.foodcont.2013.06.031>
 25. G. Spigno, L. Tramelli, D.M. De Faveri, Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics, *J. Food. Eng.* 81 (2007) 200–208.
<http://dx.doi.org/10.1016/j.jfoodeng.2006.10.021>
 26. V.L. Singleton, J.A. Rossi Jr., Colourimetry of total phenols with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic.* 16 (1965) 144–158.
 27. P. Ribéreau-Gayon, The amount of total phenolic compounds in red wines, *Chim. Anal.* 52 (1970) 627–631 (in French).
 28. S. Pérez-Magari-o, M.L. González-San José, Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening, *J. Agric. Food Chem.* 52 (2004) 1181–1189.
<http://dx.doi.org/10.1021/jf035099i>
 29. M. Del Alamo Sanza, J.A. Fernández Escudero, R. De Castro Torío, Changes in phenolic compounds and colour parameters of red wine aged with oak chips and in oak barrels, *Food Sci. Technol. Int.* 10 (2004) 233–241.
<http://dx.doi.org/10.1177/1082013204046095>
 30. Sensory Analysis: General Guidance for the Design of Test Rooms, ISO 8589:2007, International Organization for Standardization (ISO), Geneva, Switzerland.
 31. Sensory Analysis: Identification and Selection of Descriptors for Establishing a Sensory Profile by a Multidimensional Approach ISO 11035:1994, International Organization for Standardization (ISO), Geneva, Switzerland.
 32. J.P. dos Anjos, M. das Graças Cardoso, A.A. Saczk, H.S. Dórea, W.D. Santiago, A.M.R. Machado *et al.*, Evolution of the concentration of phenolic compounds in cachaça during aging in an oak (*Quercus* sp.) barrel, *J. Braz. Chem. Soc.* 22 (2011) 1307–1314.
<http://dx.doi.org/10.1590/S0103-50532011000700016>
 33. M.J. Cabrita, C. Barrocas Dias, A.M. Costa Freitas, Phenolic acids, phenolic aldehydes and furanic derivatives in oak chips: American vs. French oaks, *S. Afr. J. Enol. Vitic.* 32 (2011) 204–210.
 34. S. Canas, A.P. Belchior, A. Falcão, J.A. Gonçalves, M.I. Spranger, R. Bruno de Sousa, Effect of heat treatment on the thermal and chemical modifications of oak and chestnut wood used in brandy ageing, *Ciência Téc. Vitiv.* 22 (2007) 5–14.
 35. J.N. Boidron, P. Chatonnet, M. Pons, Effects of wood on aroma compounds of wine, *Conn. Vigne Vin.* 22 (1988) 275–294.
 36. S. Canas, M.C. Leandro, M.I. Spranger, A.P. Belchior, Low molecular weight organic compounds of chestnut wood (*Castanea sativa* L.) and corresponding aged brandies, *J. Agric. Food Chem.* 47 (1999) 5023–5030.
<http://dx.doi.org/10.1021/jf9900480>

37. S. Canas, H. Quaresma, A.P. Belchior, M.I. Spranger, R. Bruno de Sousa, Evaluation of wine brandies authenticity by the relationships between benzoic and cinnamic aldehydes and between furanic aldehydes, *Ciência Téc. Vitiv.* 19 (2004) 13-27.
38. R. Gimenez-Martínez, H. López-García de la Serrana, M. Villalón-Mir, M. Navaro-Alarcón, M. Olalla-Herrera, C. Cabrera-Vique, M.C. López-Martínez, Study of vanillin, syringaldehyde and gallic acid content in oak wood and wine spirits mixtures: Influence of heat treatment and chip size, *J. Wine Res.* 12 (2001) 175-182.
<http://dx.doi.org/10.1080/09571260120106811>
39. C. Gómez-Cordovés, B. Bartolomé, M.L. Jimeno, Identification of 2,3-dihydroxy-1-guaiacylpropan-1-one in brandies, *J. Agric. Food Chem.* 45 (1997) 873-876.
<http://dx.doi.org/10.1021/jf9605613>
40. F.P. van Jaarsveld, S. Hattingh, P. Minnaar, Rapid induction of ageing character in brandy products. Part III. Influence of toasting, *S. Afr. J. Enol. Vitic.* 30 (2009) 24-37.
41. J.L. Puech, M. Moutounet, Phenolic compounds in an ethanol-water extract of oak wood and in a brandy, *Lebensm. Wiss. Technol.* 25 (1992) 350-352.
42. V.N. Vlassov, D.S. Maruzhenkov, Application of GC/MS method for the identification of brandies and cognacs, *Analisis*, 27 (1999) 663-667.
<http://dx.doi.org/10.1051/analisis:1999136>