American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (8): 631-646, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.631.646

Phenolic Constituents, Furfuraldehyde and Antioxidant Capacity of Sugar Cane Spirit Aged in Woods Casks

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ABSTRACT

This study quantified the phenolic compounds (gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coumarin and scopoletin), furfuraldehyde and the antioxidant capacity during in 0, 1, 3, 6, 9, 12 and 18 months of non-aged cachaça and active aging of cachaça in 20 L casks of four Brazilian wood species, white peroba (*Paratecoma white peroba*), balsamo (*Myroxylon balsamum*), timborana (*Piptadenia* sp.), jaboty (*Erisma uncinatum*) and white oak (*Quercus* sp.) casks. The content of phenolic compounds and 2-furfuraldehyde were quantified by HPLC-UV. The antioxidant capacity was determined using two indirect methods: The total phenolic content by Folin-Ciocalteu assay and the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The phenolic compounds incorporated in aged cachaças after 18 months of aging were: jaboty (gallic acid and vanillin), white oak (gallic acid, vanillic acid, vanillin, syringic acid and syringaldehyde), timborana (gallic acid, vanillic acid, vanillin and coumarin), balsamo (gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde and coumarin) white peroba (gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde and coumarin). The antioxidant capacity and content of phenolic compounds both increase with increasing aging time and there is a significant correlation between them. The Brazilian woods balsam, followed by white peroba and timborana were the best substitute to replace the oak casks because their aged cachaças presented higher antioxidant capacity and phenolic profile more complex and quantitatively greater than the cachaça aged in white oak casks. This work suggests that 2-furfuraldehyde can degrade in cachaça aged in timborana, white peroba and balsamo.

Key words: Cachaça, wood maturation, phenolic compounds, antioxidant capacity, distilled beverages

INTRODUCTION

Sugar cane spirit produced in Brazil receive a typical and exclusive name of cachaça, which is produced by the distillation of fermented sugarcane juice, possesses distinctive sensory characteristics, alcoholic percentage by volume ranges from 38 to 48% at 20°C and may contain an added sugar content of up to 6 g L^{-1}, expressed in sucrose (Brazilian Ministry of Agriculture, Livestock and Food Supply, 2005).

The production of aged cachaça can be divided into four major steps: production of must, fermentation, distillation and aging (Faria, 2003). Regardless of the procedures employed in the first three steps of the production process, the new distillate has a dry, burning flavor and a somewhat unpleasant aroma. Aging is therefore essential for cachaça to acquire the desired properties of color, aroma and flavor typical of high quality distillates (Odello *et al.*, 2009). Aging is too a major source of phenols in alcoholic beverages, such as rum, wine, whiskey, brandy and cachaça have varying contents of phenolic compounds and antioxidant capacity (Mosedale and Puech, 1998; Madrera *et al.*, 2003; Goldberg *et al.*, 1999; Arnous *et al.*, 2001; Da Silva *et al.*, 2009). Phenolic compounds have biological properties such as anti-inflammatory, antiallergic, antibacterial, antimicrobial, cardioprotective and antioxidant capacity. Increasing attention has recently focused on antioxidant compounds and their possible prevention of numerous chronic diseases involving oxidative stress. Antioxidant capacity, an important mechanism against oxidative stress in the defense of living systems is the ability to capture radicals. In profusion, such radicals can induce oxidative damage inbiomolecules, resulting in atherosclerosis, aging, cancer and many other diseases (Valko *et al.*, 2006; Oliveira *et al.*, 2009). Aging and therefore the content of these phenolic compounds are affected by several factors such as the characteristics of the beverage, the species and origin of the wood used for casks, cask size, cask treatment (charring), aging time and the environment in which aging takes place (Mosedale and Puech, 1998).

In aged alcoholic beverages such as wine, brandy and cachaça, the presence of low molecular weight phenolic compounds has been reported. The phenolic compounds identified were vanillin, syringaldehyde, syringic acid, vanillic acid, gallic acid coumarin, scopoletin and furanic compounds (Madrera *et al.*, 2003; Goldberg *et al.*, 1999; Arnous *et al.*, 2001; Da Silva *et al.*, 2009; De Aquino *et al.*, 2006; Aquino *et al.*, 2005). Dias *et al.* (1998) evaluated the effect of the Brazilian woods amburana (*Amburana cearensis* (Fr. All.) A.C. Smith); bálsamo (*Myroxylon peruiferum* L.F.); jequitibá (*Cariniana estrellensis* (Raddi) Kuntze); jatobá (*Hymenaea* spp.); ipê (*Tabebuia* sp.) and of the European oak (*Quercus* sp.) on the composition of sugarcane spirits aged for six months and found that each type of wood introduced specific phenolic compounds into the spirits. Brazilian woods have been evaluated in previous works as an alternative to replace the oak used for the aging of cachaça. Since Brazil has a large and exotic flora while white oak is imported from the United States and Europe and its supply is limited. For which reason second-hand or third-hand casks are normally used. In a sensory evaluation, amendoim (*Pterogyne nitens*), pereiro (*Aspidoesperma pyrifolium*) and jatoba (*Hyemenaea stigonocarpa*) casks were found to be the best candidates to replace oak (*Quercus* sp.). The total phenols in these aged cachaças ranged from 80-300 ppm (Faria *et al.*, 2003). Recent studies determined the phenolic compound profile of extracts of cachaça and sawdust of different Brazilian wood species jatobá (*Hyemenaea courbaril*), cabreúva-parda (*Murocarpus frondosus*), amendoim (*Pterogyne* sp.), canela-sassafrás (*Ocotea pretiosa*), pequí (*Caryocar brasiliense*) and oak samples of USA and Europe (*Quercus* sp.). These study found that coumarin and catechin were the most abundant compounds in extracts of cachaça and Brazilian wood species sawdust while oak extracts were richer in syringaldehyde and coniferaldehyde (Da Silva *et al.*, 2009). Extracts of cachaça and sawdust of wood specie amendoim (*Pterogyne* sp.) and jatobá (*Hyemenaea courbaril*) showed higher antioxidant efficiency and sensorial improvement than extracts of canela-sassafrás (*Ocotea pretiosa*), castanheira (*Castanea* sp.), ipê (*Tabebuia chrysotricaha*), jatobá (*Hyemenaea courbaril*), louro-canela (*Aniba parviflora*) and oak samples of USA and Europe (*Quercus* sp.) (Cardoso *et al.*, 2008). Aging is a stage of the production process that is important for the extraction of phenolic compounds, enhancing the sensory qualities and antioxidant capacity of cachaça which can has nutraceutical properties if consumed in moderation. However, the phenolic compounds and

antioxidant capacity of cachaça during effective aging for 18 months in casks of species of wood white peroba (*Paratecoma white peroba*), balsamo (*Myroxylon balsamum*) timborana (*Piptadenia* sp.) and jaboty (*Erisma uncinatum*) casks have so far not been quantified and since the majority of studies have only analyzed wood sawdust extracts which represents only a qualitative evaluation of these compounds in cachaça. Moreover, no studies have focused on possible alterations in furfural content during aging in these wood casks.

This study quantified the phenolic compounds (gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coumarin and scopoletin), furfuraldehyde and the antioxidant capacity during 18 months of non-aged cachaça and active aging of cachaça in white peroba (*Paratecoma white peroba*), balsamo (*Myroxylon balsamum*), timborana (*Piptadenia* sp.), jaboty (*Erisma uncinatum*) and white oak (*Quercus* sp.) casks. Correlation between phenolic composition and antioxidant capacity was also assessed.

MATERIALS AND METHODS

This study was conducted in period of October 23, 2007 to October 5, 2010. The aging process of the samples was conducted at Laboratories of the derivatives cane sugar, Technology Center, Federal University of Alagoas, Brazil. The chemical analyses of samples were conducted at the Laboratory of Chemical Instrumental of Federal Institute for Education, Science and Technology of Alagoas, Brazil.

Materials: Cachaça was purchased from a local distillery, J. Gomes Neto-Me (Joaquim Gomes, AL, Brazil). New wooden casks of 20 L for aging cachaça were purchased from Brazilian manufacturers and they were charred during their manufacture. All the woods used in the production of the casks were identified botanically by macroscopic and microscopic methods for the examination of wood anatomy in the Laboratory of Wood and Plant Products at the IPT-Institute for Technological Research (São Paulo, Brazil) which certified the samples of white oak (*Quercus* sp.) and the Brazilian woods species white peroba (*Paratecoma peroba*), balsamo (*Myroxylon balsamum*), timborana (*Piptadenia* sp.) and jaboty (*Erisma uncinatum*). The names of woods are used in accordance with BS EN 13556:2003 of the British Standards Institution, except for timborana whose trivial name was not found in any international standard and we used the trade name given in Brazil (The British Standards Institution Staff, 2003). The empty casks had the following dimensions: 33 cm diameter base, 28 cm diameter top and 44 cm height.

Each cask was filled with 20 L of cachaça and stored upright for 18 months in a poorly lit room, at a temperature of 19 to 26.5°C and relative humidity of 67 to 80%. A total of 42 cachaça samples were analyzed, comprising the non-aged cachaça and five types of cachaça aged in casks of different wood species evaluated at 0, 1, 3, 6, 9, 12 and 18 months of aging. Each sample of aged cachaça was identified with the name of the wood species of its cask, followed by the number of months of aging, e.g., balsamo18, white peroba1 and balsamo1-18 (refer to samples collected in months 1, 3, 6, 9, 12 and 18). The samples were filtered through disposable M illex syringe filters from Sigma-Aldrich Brazil Ltda. (São Paulo, Brazil) with a diameter of 25 μm and a 0.45 mm glass fiber membrane attached to acrylic resin. They were then stored in glass jars and refrigerated at 5 to 7°C in the dark.

Reagents and chemicals: All the solvents employed were of HPLC grade purchased from Sigma-Aldrich Brazil Ltda. (São Paulo, Brazil) and used as received. Purified water was produced by a

Millipore Milli-Q system (Bedford, MA, USA). The standards and analytical grade reagents which were purchased from Sigma-Aldrich Brazil Ltda., were gallic acid (3,4,5-trihydroxybenzoic acid), syringic acid (3,5-dimetoxy-4-hidroxybenzoic acid), vanillic acid (4-hidroxy-3-metoxybenzoic acid), vanillin (4-hydroxy-3-methoxybenzaldehyde), syringaldehyde (3,5-dimetoxy-4-hydroxybenzaldehyde), scopoletin (7-hydroxy-6-methoxy-2H-chromen-2-one), coumarin (2H-chromen-2-one), 2-furfuraldehyde (2-furancarboxaldehyde), DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu phenol reagent 2N, used without prior purification.

HPLC/UV analysis: The phenolic compounds and furfural were assayed by High Performance Liquid Chromatography (HPLC) using a Shimadzu HPLC system (Kyoto, Japan) composed of the following modules: SCL-10 AVP system controller, SPD-10 AVP UV-VIS detector, LC-10ADVP 2-stage pump, a manual Rheodyne 0495 injector with a 20 μL loop and Class-VP data acquisition software. The phenolic compounds and furfural were separated in a Shimadzu Shim-pack CLC-ODS (M) column (Tosoh, Japan), with 4.6 mm i.d. (inside diameter)×15 inches length and the analysis was performed by reversed phase.

The HPLC analysis to quantify the phenolic compounds and furfural was performed using a modified version of the methodology previously developed for the analyses of cachaça samples (De Aquino *et al.*, 2006). Different mobile phases were evaluated and the best condition for separation was obtained with an isocratic elution of a mixture of water/acetic acid/methanol solvents (93.1/1.9/5.0%). Elution was performed at room temperature and at a constant flow rate of 1.25 mL min^{-1}, resulting in a total elution time of 75 min. Spectral data for all peaks were accumulated in the range 200-400 nm using SPD-10AVP UV-VIS detector. The structural identifications were made by direct comparisons of retention time, UV spectra of standards and by spiking the samples with the standards. Standard chromatograms were plotted at 280 nm.

The analytes were quantified by the external standard method, using eleven standard multi-element solutions containing gallic acid (0.025-57.1 mg L^{-1}), furfural (0.012-28.6 mg L^{-1}), vanillic acid and syringic acid (0.099-228.6 mg L^{-1}), vanillin and coumarin (0.05-114.3 mg L^{-1}), syringaldehyde (0.05-228.6 mg L^{-1}) and scopoletin (0.124-285.7 mg L^{-1}). The analytical curves ($y = a+bx$) were built by linear regression of the data obtained for the mean peak area of each analyte after triplicate injection of 20 μL of each of the eleven multi-element standard solutions (ethanol 50% v/v adjusted to pH 4.3). Thereafter were calculated the precision, accuracy and Limits of Detection (LOD) and quantification (LOQ). The precision or measure of spread was determined by relative standard deviation ($RSD = s/x \times 100$). Accuracy was determined through method of standard additions obtained the values of experimental versus theoretical concentrations. The limits were determined from data obtained from the analytical curves, the limits of detection ($LOD = 3\ s_B/a$) and quantification ($LOQ = 5\ s_B/a$), where s_B is the standard deviation of intercept and a is a slope of least square line. Injections of 20 μL in HPLC were done in triplicate of each of the 42 cachaças samples. The SPC-sum of the phenolic compounds (mg L^{-1}) was determined by calculating the sum of the concentration of each phenolic compound analyzed by HPLC.

Total phenolic content: Folin-Ciocalteu method: The total phenolic contents of the samples were determined in triplicate in Gallic Acid Equivalents (GAE) using the modifications of Folin-Ciocalteu method (Singleton and Rossi, 1965). The procedure consisted of the following: 50 μL of cachaça sample, 3.7 mL of distilled water, 250 μL of Folin-Ciocalteu reagent 2 N and 1 mL of sodium carbonate 20% (w/v) were placed in calibrated test tubes. The test tubes were shaken to homogenize their contents and allowed to rest in the dark for 30 min to stabilize the reaction. The absorbance at 750 nm wasdetermined in a Shimadzu UV mini-1240 spectrophotometer in quartz

cells with a 10-mm optical path. A calibration curve was prepared with gallic acid solutions in the concentration range of 0 to 1000 mg L^{-1} and the results were expressed in milligrams as Gallic Acid Equivalents (GAE) per liter of cachaça (mg_{GAE} L^{-1}). The following calibration curve, was used to calculated the concentration of total phenolic (mg_{GAE} L^{-1}): Total phenolic mg $_{GAE}$ L^{-1} = 1068.6× Abs_{750nm} + 26.54, correlation coefficient r = 0.99351.

Inhibition of DPPH radical: The antioxidant capacity was determined by the modified DPPH method (Scherer and Godoy, 2009; Aoshima *et al.*, 2004). Initially, a control solution was prepared of DPPH (40 μL mL^{-1}) in methanol. Reaction mixtures were prepared with 140 μL of cachaça sample, 160 μL of methanol and 2.7 mL of the DPPH control solution. After the DPPH control solution was added, counting of the reaction time started. After 30 min of reaction time, the absorbance at 517 nm was determined in a Shimadzu UVmini-1240 spectrophotometer in quartz cells with a 10 mm optical path. The results were expressed as I-inhibition of the DPPH radical (%) calculated as follows:

$$I\% = [(Abs_0-Abs_1)/Abs_0]\times100$$

where, Abs_0 is the absorbance of the DPPH control solution and Abs_1 is the absorbance of the reaction mixture in 30 min of reaction.

Statistical analysis: The statistical analysis was performed using Statistica™ 5.0 software (Stat Soft, Inc., USA). In the study the experimental design was development with two factorial designs, which are: wood species/genres botanical (varying in six levels with five wood botanical species/genres and non-aged cachaça with control) and aging times (varying in seven levels of aging times 0, 1, 3, 6, 9, 12 and 18 months). The two-way analysis of variance to evaluate the effects of on all variables, which are: phenolic compounds, furfuraldehyde and antioxidant capacity (total phenolic and inhibition of DPPH radical). Tukey's test was employed to evaluate the difference between the means, with a significance level ($p<0.05$) (Miller and Miller, 2005).

In addition, a multivariate analysis by Principal Components Analysis (PCA) and a Hierarchical Cluster Analysis (HCA) were performed to evaluate the similarity among the cachaça samples based on the formation of different groups according to the wood species of the casks used in the aging process. The experimental data were compiled to generate a final matrix (42×10) of 42 factors (samples of non-aged cachaça and cachaça aged in fivedifferent woods, collected at 0, 1, 3, 6, 9, 12 and 18 months) and 10 variables (gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coumarin, furfural, SPC, total phenols and inhibition of the DPPH free radical). Each sample was analyzed in triplicate and the data were expressed as mean and the error bars as±standard deviation (Std). These data were previously averaged and the scales were standardized to ensure that all the variables would contribute equally to the model, independently of the scale on which they were measured. The complete-linkage method and Euclidean distances were used to generate the dendogram by the HCA method (Miller and Miller, 2005).

RESULTS AND DISCUSSION

HPLC/UV analysis: The HPLC method applied in this work was validated and showed good precision, accuracy and linearity for the standard solutions. All calibration curves presented correlation coefficient higher than 0.9996. All compounds presented low detection limits, except scopoletin. The all parameters of validation and retention time for each phenolic compound and furfuraldehyde under investigation are presented in Table 1.

Table 1: Parameters of validation of the HPLC method: retention time (rt), parameters of analytical curves (b-slope and a-intercept), correlation coefficients (r), the working range, precision (RSD), accuracy and Limits Of Detection (LOD) and Limits Of Quantification (LOQ)

	rt (min)	b	a	r	Range (mg L^{-1})	RSD (%)	Accuracy (%)	LOD (mg L^{-1})	LOQ (mg L^{-1})
Gallic acid	3.00	4.0137×10^{4}	-1.3926×10^{3}	0.9996	0.025-57.1	4.84	100.82	0.31	0.51
Furfural	5.15	1.0077×10^{5}	-1.3926×10^{3}	0.9999	0.012-28.6	3.10	100.97	0.05	0.08
Vanillic acid	16.54	2.7857×10^{4}	7.4852×10^{2}	0.9999	0.09-228.6	6.46	105.01	0.29	0.48
Vanillin	24.10	6.7224×10^{4}	-5.8608×10^{3}	0.9999	0.09-228.6	7.42	101.36	0.16	0.27
Syringic acid	28.29	4.7225×10^{4}	1.9154×10^{3}	0.9999	0.09-228.6	6.49	97.27	0.58	0.97
Syringaldehyde	42.82	3.4025×10^{4}	-1.3448×10^{4}	0.9998	0.05-228.6	7.71	99.71	0.56	0.93
Coumarin	57.14	6.6227×10^{4}	-1.5372×10^{4}	0.9999	0.05-114.3	3.75	104.03	0.41	0.68
Scopoletin	66.30	1.6339×10^{4}	-3.0771×10^{3}	0.9998	0.12-285.7	3.10	104.69	1.07	1.79

Calibration plots are expressed as linear regression equations (y = a + bx), where, y is the peak area and x is the analyte concentration (mg L^{-1})

The representative chromatograms were obtained of standard multi-element solution (Fig. 1a), non-aged cachaça (Fig. 1b) and cachaça aged in casks of balsamo (Fig. 1c), jaboty (Fig. 1d), white oak (Fig. 1e), white peroba (Fig. 1f) and timborana (Fig. 1g).

Each phenolic compound and furfuraldehyde was quantified based on the equations of the analytical curves and their respective peak areas of the HPLC chromatograms. Under the experimental conditions of HPLC analyses were quantified the gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coumarin and scopoletin and furfuraldehyde, in non-aged cachaça and cachaça aged in balsamo, jaboty, white oak, white peroba and timborana casks evaluated at 0, 1, 3, 6, 9, 12 and 18 months of aging, as shown in Fig. 2.

Gallic acid: Figure 2a shows the mean concentrations of gallic acid in non-aged cachaça and in cachaça undergoing the 18-month aging process. The non-aged cachaça showed a constant mean gallic acid concentration of 1.07 mg L^{-1}. The cachaça containing the highest concentrations of gallic acid were balsamo 18 (49.39 mg L^{-1}) followed by timborana 18 (8.95 mg L^{-1}). The cachaça aged in the balsamo cask showed a rapid increase in the concentration of gallic acid starting in the first month of aging. The increase in the concentration of gallic acid may be due to the hydrolysis of wood tannins as has been reported for cider brandy (Mangas *et al.*, 1996). Cachaça aged in casks of jaboty 1-18, white oak 1-18 and white peroba 1, 12 and 18 showed no significant increase in the concentration of gallic acid when compared with non-aged cachaça. Among the phenolic compounds analyzed in this study, gallic acid presented the largest quantities (percentage) in non-aged cachaça (35.55%), followed by cachaças aged in white oak 18 (37.05%), jaboty 18 (55.75%) and balsamo 18 (71.90%). Moreover, 17.85% of gallic acid ranked second among the compounds found in cachaça timborana 18. De Aquino *et al.* (2006) found in commercial aged cachaça mean concentrations of gallic acid of 0.6293 mg L^{-1} for small producers and 0,1528 mg L^{-1} for export product, these results is the same order of magnitude as that found for gallic acid in cachaça aged in oak casks in this study. Da Silva *et al.* (2009) found mean of gallic acid 6.6 mg L^{-1} in oak USA sawdust extracts. This higher amount of gallic acid found may be due to sawdust have a larger contact area than the wooden casks, providing a greater extraction of phenolic compounds. Goldberg *et al.* (1999) found 3.23 mg L^{-1} of the gallic acid in cognac on previous study. However, the aging time for the cognac was over 10 years in *Limousin* Oak casks. In a previous work, aged red wine from Greece aged in oak casks showed 288.4 mg L^{-1} of gallic acid, which is equivalent to

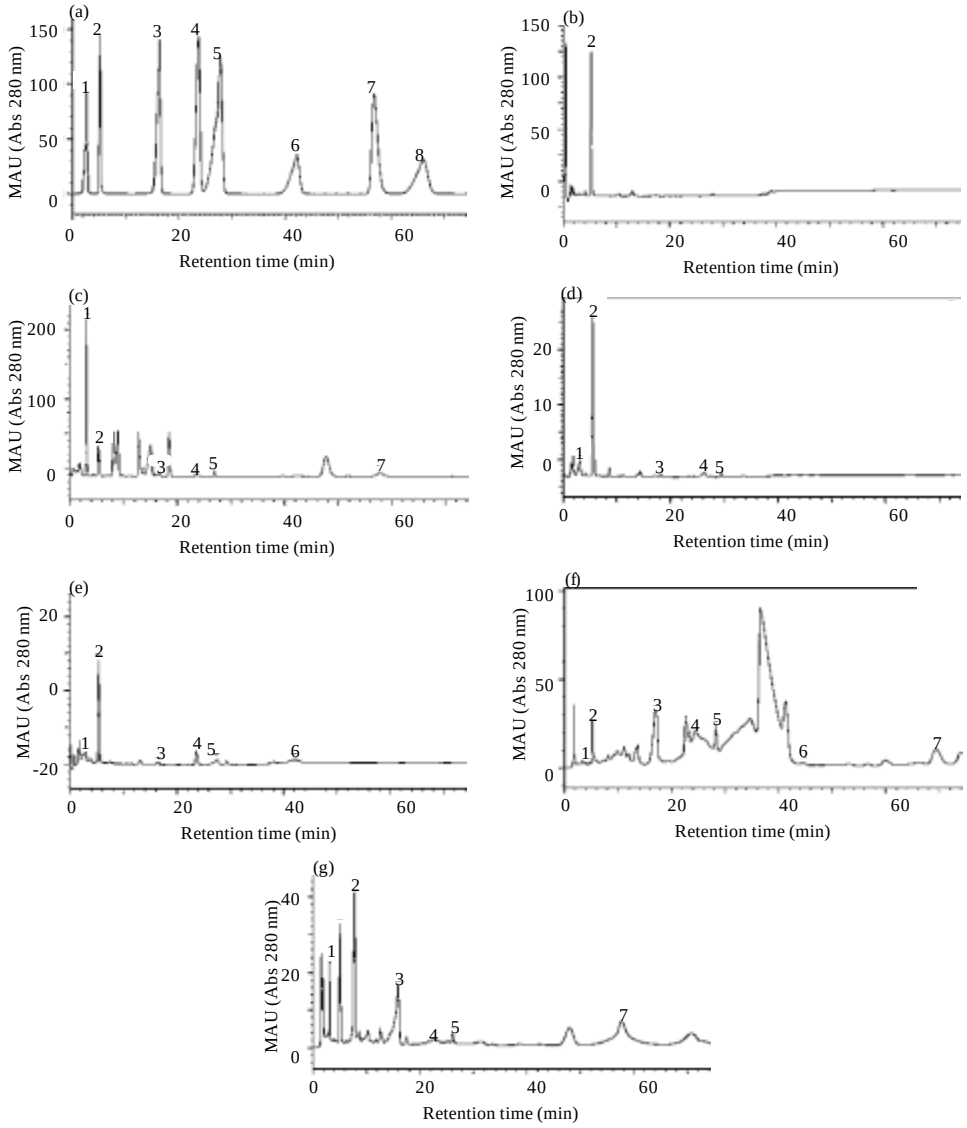

Fig. 1: HPLC chromatograms at 280 nm of phenolic compounds and furfuraldehyde of (a) multi-element standard solution, (b) non-aged cachaça, and cachaça aged in (c) balsamo 18, (d) jaboty 18, (e) white oak 18, (f) white peroba, (g) timborana. The identified peaks were (1) gallic acid, (2) 2-fufuradehyde, (3) vanillic acid, (4) vanillin, (5) syringic acid, (6) syringaldehyde, (7) coumarin and (8) scopoletin

85.3% of the total phenolic acids in its composition (Arnous *et al.*, 2001). Most of gallic acid found in red wines aged in oak barrels comes from the grape itself, with an increase of its content during aging in oak barrels (Del Souza *et al.*, 2004).

The variations found in the content of phenolic compounds analyzed may occur due to numerous factors, such as ageing time, botanical species of wood casks, construction method of the casks, the casks treatment, environmental conditions of temperature and humidity, where it is made the process of ageing, the chemical characteristics of distillate, etc. (Mosedale and Puech, 1998).

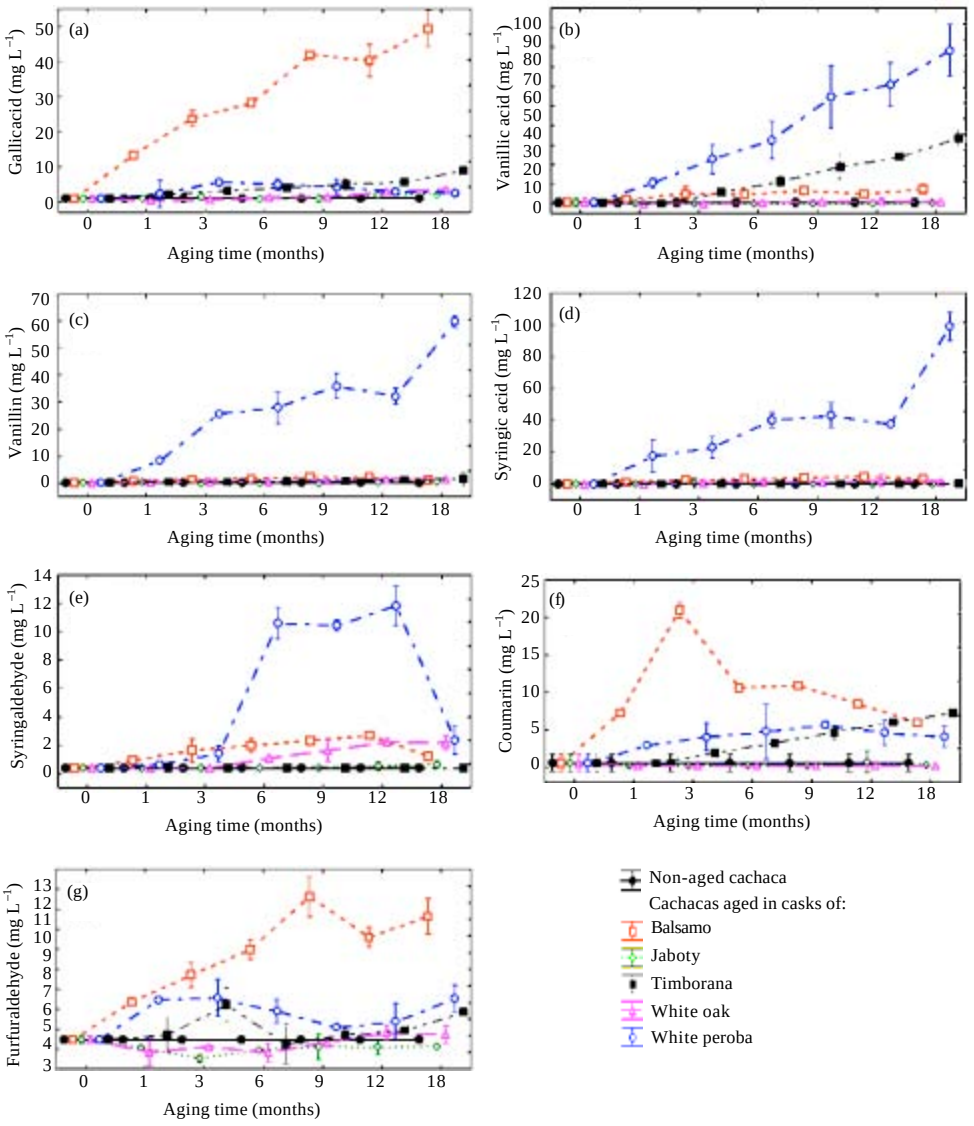

Fig. 2: Mean concentration (mg L^{-1}) of: (a) gallic acid, (b) vanillic acid, (c) vanillin, (d) syringic acid, (e) syringaldehyde, (f) coumarin, (g) furfuraldehyde, in non-aged cachaça and cachaça aged for 18 months in balsamo, jaboty, white oak, white peroba, and timborana casks. The bars represent the standard deviation of three replicates

Vanillic acid: Non-aged cachaça contained a mean concentration of vanillic acid of 0.61 mg L^{-1}, did not differing significantly from cachaça aged in casks of jaboty 1-18 and white oak 1-18 (Fig. 2b). The cachaças that showed difference significantly of the non-aged cachaça were white peroba 1-18, timborana 3-18 and balsamo 1-18. The concentration of vanillic acid was highest in white peroba 18 (78.38 mg L^{-1}), followed by timborana 18 (33.71 mg L^{-1}) and balsamo 18 (7.58 mg L^{-1}). Vanillic acid was the phenolic compound analyzed found in the largest quantity in cachaça timborana 18 (67.25%) and the second largest quantity in non-aged cachaça (0.61 mg L^{-1}-20.27%), followed by cachaça aged in balsamo 18 (7.58 mg L^{-1}-11.04%) and

white peroba 18 (78.38 mg L^{-1}-31.74%). Previous works found 0.9 mg L^{-1} of vanillic acid concentrations in commercial aged cachaça and 0.18 mg L^{-1} in oak extract (De Aquino *et al.*, 2006; Da Silva *et al.*, 2009). Most of the commercial cachaças in Brazil are aged in oak casks, which explain the low values for syringic acid.

Vanillin: Non-aged cachaça showed a mean vanillin concentration below the limit of quantification 0.27 mg L^{-1} and only cachaça aged for up to 18 months in the white peroba 1-18 cask showed a significant increase in vanillin concentration of 8.88 to 59.84 mg L^{-1}, as indicated in Fig. 2c. In general, it is well established that benzoic acids such as vanillic acid and syringic acid, result from cinnamic aldehyde, vanillin and syringaldehyde, respectively, by means of a β-oxidation process, and the subsequent oxidation of the benzoic aldehyde formed. However, a decreased in the corresponding cinnamic aldehydes vanillin was not found; on the contrary, the concentration of vanillin increased during aging. This fact might be explained on the basis of the more intensive lignin hydrolysis in relation to the oxidation process of aldehydes which promotes the accumulation of cinnamic alcohols and aldehydes, likewise in previous work (Mangas *et al.*, 1996). The cachaças aged in balsamo 1-18, jaboty1-18, timborana 1-18 and white oak 1-18 did not show a significant increase in vanillin concentration compared to that of non-aged cachaça. A previous study reported 1.31 to 1.54 mg L^{-1} of vanillin in commercial aged cachaça (De Aquino *et al.*, 2006). Da Silva *et al.* (2009) quantified 0.077 mg L^{-1} of vanillin in oak sawdust extracts. In previous studies, variations in vanillin concentrations were found in several sawdust extracts of Brazilian wood species and oak, ranging from 0 to 0.098 mg L^{-1} (Da Silva *et al.*, 2009). In another study, Dias *et al.* (1998) found 14.91 mg L^{-1} of vanillin in cachaça aged for 6 months in a 20 L balsamo cask. As mentioned earlier, the variations found in the content of phenolic compounds analyzed may occur due to numerous factors. Vanillin has a limit of odor detection of about 0.1 mg L^{-1} in 40% ethanol and has a sweetish vanilla aroma (Singleton, 1995). Only cachaça aged in white peroba presented a vanillin content well above the limit of odor detection. This wood is recommended for use in the cachaça aging process because of the desirable sensory characteristics vanillin confers on alcoholic beverages.

Syringic acid: As indicated in Fig. 2d, the concentration of syringic acid in non-aged cachaça was slightly below the detection limit of 0.97 mg L^{-1} of the analytical method. Only cachaça aged in white peroba 1-18 showed a significant increase in syringic acid concentration. Among the phenolic compounds analyzed in this work, syringic acid was found in the largest amounts in cachaça aged in white peroba 18 (99.20 mg L^{-1}-40.17%). In a previous study, De Aquino *et al.* (2006) found syringic acid concentrations varying from 1.94 to 2.1 mg L^{-1} in samples of aged cachaça. In contrast, not detected syringic acid in any extract of sawdust from the various wood species analyzed (Da Silva *et al.*, 2009).

Syringaldehyde: Non-aged cachaça showed a mean concentration syringaldehyde below the limit of quantification of 0.93 mg L^{-1} (Fig. 2e). Cachaça aged in white oak 6-18, balsamo 3-18 and white peroba 3-18 casks showed a significant increase in syringaldehyde concentration when compared with non-aged cachaça. The concentration of syringaldehyde increased significantly in cachaça aged in the white peroba cask up to the 12th month of aging, declining rapidly thereafter. This may be explained, by the conversion of syringaldehyde to syringic acid, as indicated by the increase in the concentration of syringic acid starting from the 12th month of aging in the white peroba cask (Mosedale and Puech, 1998). The concentration of the syringic acid increased during aging and

a decrease in the corresponding cinnamic aldehydes was found. Cachaças aged in jaboty 1-18 and timborana 1-18 casks do not incorporate significant amounts of syringaldehyde when compared with non-aged cachaça. Among the phenolic compounds analyzed here, syringaldehyde was present in the second highest amount in cachaça aged in jaboty 18 (0.65 mg L^{-1}-16.62%) and white oak 18 (2.18 mg L^{-1}-22.31%) casks. There are larger amounts of syringaldehyde in oak extracts than in Brazilian wood extracts, as observed in previous studies (Da Silva *et al*., 2009). De Aquino *et al*. (2006) found 5.24 to 6.26 mg L^{-1} of syringaldehyde in commercial cachaça. Da Silva *et al*. (2009) quantified 0.14 to 0.21 mg L^{-1} of syringaldehyde in oak extracts and 0.22 to 3 mg L^{-1} in commercial cachaça.

Coumarin: Figure 2f indicate that non-aged cachaça contained a mean concentration of coumarin below the limit of quantification of 0.68 mg L^{-1}. Only cachaça aged in timborana 3-18, balsamo 1-18 and white peroba 1-18 casks incorporated a significant amount of coumarin during the aging process when compared with non-aged cachaça. The coumarin concentrations determined in aged cachaças were 7.27 mg L^{-1} in timborana 18, 5.97 mg L^{-1} in balsamo 18 and 4.08 mg L^{-1} in white peroba 18. Cachaça aged in the balsamo cask showed increasing concentrations of coumarin up to the 3rd month of aging, followed by decreasing concentrations up to the 18th month of aging. . The coumarin concentration of cachaça aged in the balsamo 3-9 cask exceeded the maximum limit of 9.52 mg L^{-1} established by the Codex Alimentarius (equivalent to 10 mg kg^{-1}, considering a cachaça density of 0.9521 mg mL^{-1}) while all other cachaça samples presented lower coumarin concentrations (Sproll *et al*., 2008). The coumarin concentration in cachaça aged in balsamo casks should be monitored to avoid exceeding the permissible limit for coumarin. However, if aging is carried out in larger barrels (200-700 L) whose contact areas are 3.36 to 5.9 times smaller than that of a 20 L cask, the extractive capacity and concentration of coumarin will be lower than in 20 L casks. The contact areas of the casks were calculated based on the ratio of cachaça volume-to-cask contact surface area of approximately 25.87 L m^{-2} for a 20 L cask, 86.93 L m^{-2} for a 200 L barrel and 152.67 L m^{-2} for a 700 L cask. Therefore, all the casks studied for aging cachaça for 18 months, regardless of their size, are suitable because their coumarin extraction capacity lies within the limit established by the Codex Alimentarius. Brazilian wood sawdust extracts contain larger amounts of coumarin than oak sawdust extracts, conform previous work (Da Silva *et al*., 2009). This observation was confirmed in our study, except for cachaça aged in the jaboty cask, which showed low amounts of coumarin.

Scopoletin: The non-aged cachaça and all aged cachaça showed scopoletin concentrations lower than the 1.07 mg L^{-1} detection limit of the HPLC analysis method. Coumarin and scopoletin have been proposed as parameters for measuring the degree of aging of alcoholic beverages. However, a recent study detected coumarin and scopoletin in a hydro alcoholic extract containing caramel food coloring used in the beverage industry, indicating that coumarins cannot be used as aging markers (Arnous *et al*., 2001; Izquierdo *et al*., 2000).

Furfuraldehyde: Non-aged cachaça presented a mean concentration of 2-furfuraldehyde of 4.48 mg L^{-1} (Fig. 2g). This furfural contained in non-aged cachaça may be formed: During harvesting, if the sugarcane is burned before harvesting; during distillation, if the sugar cane wine contains residual sugars or fragments of bagasse during heating; observed in previous studies (Madrera *et al*., 2003). Cachaça aged in white oak 1-18 and jaboty 1-18 casks did not show

difference of 2-furfural content during aging when compared with non-aged cachaça. In wine, furfuraldehyde accumulates in up to 6-8 months, after 10 months they degrade extensively through your reduction to the corresponding alcohol by biological mechanisms or chemical reduction (Cerdan *et al*., 2004). However, this was observed for timborana and white peroba up to 3 months of aging and for balsamo up to 9 months of aging, but after 12 months the furfuraldehyde concentrations begin increases again. Cachaça with highest 2-furfuraldehyde concentrations was found in the balsamo 9 (11.63 mg L^{-1}), white peroba 3 (6.56 mg L^{-1}) and timborana 3 (6.22 mg L^{-1}) casks. The 2-furfuraldehyde formed during aging had your concentration varieties depended on the type of wood. The concentrations of 2-furfural in non-aged cachaça and aged cachaça were well below the maximum limit established by Brazilian legislation, which allows a maximum of 50 mg L^{-1} in anhydrous alcohol (sum of furfural plus 5-hydroxymethylfurfural).

Sum of Phenolic Compounds (SPC): Figure 3a depict the results of the SPC = [gallic acid] + [vanillic acid] + [vanillin] + [syringic acid] + [coumarin] + [syringaldehyde]. Non-aged cachaça showed a SPC of 3.01 mg L^{-1} and cachaça aged in white oak 1-18 and jaboty 1-18 casks showed no significant difference in the SPC in relation to non-aged cachaça. However, cachaça aged in white peroba 1-18, balsamo 1-18 and timborana 6-18 casks showed higher SPC than non-aged cachaça. The highest SPC in aged cachaça was found in white peroba 18 (246.96 mg L^{-1}), followed by balsamo 18 (68.69 mg L^{-1}) and timborana 18 (52.40 mg L^{-1}). Considering all the cachaçasamples

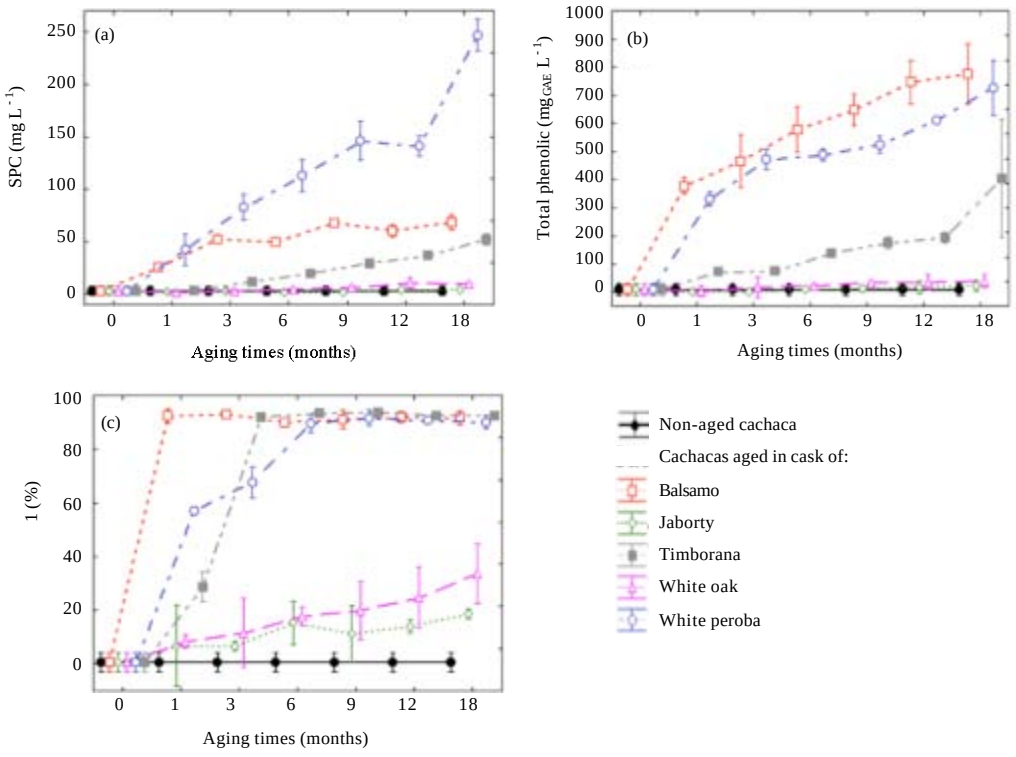

Fig. 3: Mean concentration (mg L^{-1}) of: (a) SPC, (b) Total phenolic, (c) I, in non-aged cachaça and cachaça aged for 18 months in balsamo, jaboty, white oak, white peroba and timborana casks. The bars represent the standard deviation of three replicates

analyzed here, the SPC showed a strong correlation (r) with vanillic acid (r = 0.927), vanillin (r = 0.931), syringic acid (r = 0.925) and syringaldehyde (r = 0.726); a moderate correlation with coumarin (r = 0.468) and a low correlation with gallic acid (r = 0.268).

Total phenolic: The results of the analysis of total phenolic by the Folin-Ciocalteu method are represented in mg_{GAE} L^{-1} in Fig. 3b. Non-aged cachaça showed the mean total phenolic concentration of 9.26 mg_{GAE} L^{-1} and the concentration in cachaça aged in white oak 1-18 and jaboty1-18 casks did not differ significantly from non-aged cachaça. In contrast, cachaça aged in timborana 6-18, balsamo 1-18, white peroba 1-18 casks showed a higher total phenolic concentration than non-aged cachaça. This result indicates that the botanical origin of wood can affect the phenolic composition of aged cachaças and the effect due this aspect have been reported before for aged mescal and wine (Avila-Reyes *et al.*, 2010). It was observed that the phenolic content increased continuously for cachaças aged in timborana, balsamo and white peroba. The highest total phenolic concentrations found in aged cachaça were in balsamo 18 (776.58 mgGAE L^{-1}), followed white peroba 18 (726.89 mg_{GAE} L^{-1}) and timborana 18 (405.38 mg_{GAE} L^{-1}). Conversely, Da Silva *et al.* (2009) reported that several oak extracts showed total phenolic content varying from 236 to 488 mg_{GAE} L^{-1}, i.e., much higher than the value found in the present study, which was 33.78 mg_{GAE} L^{-1} for cachaça aged in the cask of white oak 18. This discrepancy can be explained by their use of oak extracts while in present study; aging was carried out directly in a white oak cask. Were found to be strongly correlated the results of total phenols and gallic acid (r = 0.735), syringaldehyde (r = 0.736), coumarin (r = 0.739) and SPC (r = 0.811), while the results of total phenols and vanillic acid (r = 0.629), vanillin (r = 0.582) and syringic acid (r = 0.569) were moderately correlated.

Inhibition of DPPH radical (I): Figure 3c indicate that non-aged cachaça showed a mean inhibition of DPPH radical of 0.83%, i.e., very low inhibition of DPPH. All the aged cachaça samples showed higher ability to inhibit DPPH radical than the non-aged cachaça. The samples with the highest values of I were timborana 18 (92.59%) followed by balsamo 18 (91.74%), white peroba 18 (90.14%), white oak 18 (33.78%), and jaboty 18 (18.74%). These results are consistent with reports inthe literature, which state that, on average, Brazilian wood sawdust extracts have a higher antioxidant capacity than of oak extracts (Cardoso *et al.*, 2008). The profile of the antioxidant capacity is affected by the origin botanical of wood and time of aging. The ability to inhibit DPPH radical increases in line with time spent in contact with wood. It is believed that the process of maturing in oak cask is characterized by diffusion of compounds from within the wood. A strong correlation was also found between I and total phenols (r = 0.829) and coumarin (r = 0.767), as well as a moderate correlation between I and gallic acid (r = 0.569), vanillic acid (r = 0.601), vanillin (r = 0.435), syringic acid (r = 0.407), syringaldehyde (r = 0.569), and SPC (r = 0.667). The correlation of I and SPC is moderate, because each compound phenolic individually has a antioxidant capacity different of other. They can have some synergism or antagonistic effect between them, modifying the antioxidant capacity of the complex mixture of compounds phenolics. In aged cachaça may have been the synergism between phenolic compounds, providing a higher antioxidant capacity, reducing the correlation between these factors, in line with observations in previous work (Cardoso *et al.*, 2008). The discordance between SPC and I is related,

mainly, with cachaças aged in white peroba and balsamo, while the cachaça aged in white peroba showed higher SPC than the balsamo, it showed higher I than the white peroba. This fact suggest that the gallic acid, phenolic compound found in higher concentration in balsamo, it have a higher DPPH free radical scavenging capacity than the others acids and aldehydes phenolics, because it have three hydroxyl groups. These suggest that the phenolic composition rather than the concentration, could determinate the antioxidant capacity in the cachaça aged in balsamo cask.

Multivariate analysis: Based on Principal Component Analysis (PCA), it was possible to separate the aged cachaça into independent groups, as illustrated in Fig. 4. Increasing the aging time provided a detachment, at different angles, of aged cachaça in relation to non-aged cachaça. The sum of the first three principal components (PC1 62.17, PC2 25.84 and PC3 5.48%) represents 93.49% of the total variability. Taking as reference the non-aged cachaça, the aged cachaça that most contributed to the principal components PC1 and PC2 were cachaça aged in white peroba, followed by balsamo, jaboty, white oak and timborana casks.

The variables that most contributed to PC1 were: Total phenols, followed by SPC, syringaldehyde, vanillic acid, vanillin, syringic acid, furfural, coumarin, gallic acid and I (Fig. 5). The variables that most contributed to PC2 were, in descending order: gallic acid, furfural, vanillin, syringic acid, vanillic acid, coumarin, SPC, I%, total phenols, syringaldehyde.

Based on the Hierarchical Cluster Analysis (HCA), seven major groups were identified and differentiated as a function of the similarities between all the variables, comprising non-aged cachaça samples and the cachaças samples aged for 18 months, as shown by the dendogram in Fig. 6. The seven groups formed were: group 1 (non-aged cachaça), group 2 (jaboty 1-9 and white oak 1-3), group 3 (jaboty 12-18 and timborana 1), group 4 (white oak 6-18), group 5 (timborana 3-18, balsamo 1 and white peroba 1), group 6 (balsamo 3-18) and group 7 (white peroba 3-18). In general, it was found that, starting from the 3rd month of aging; it was possible to classify aged cachaça into groups. The exceptions to this rule were cachaça aged in casks of white oak 1-3 and jaboty 1-9 which showed slow and low extraction and decomposition of wood compounds, since their composition was very similar to that of non-aged cachaça.

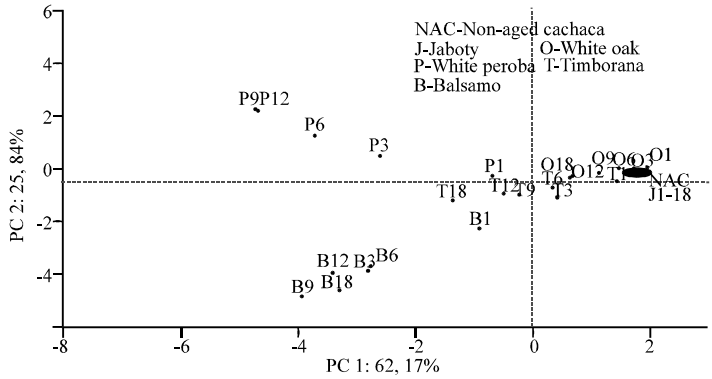

Fig. 4: Analysis of principal components of analytical data: Biplot with PC1 and PC2 showing a projection of the cachaças samples on the plane defined by two principal components

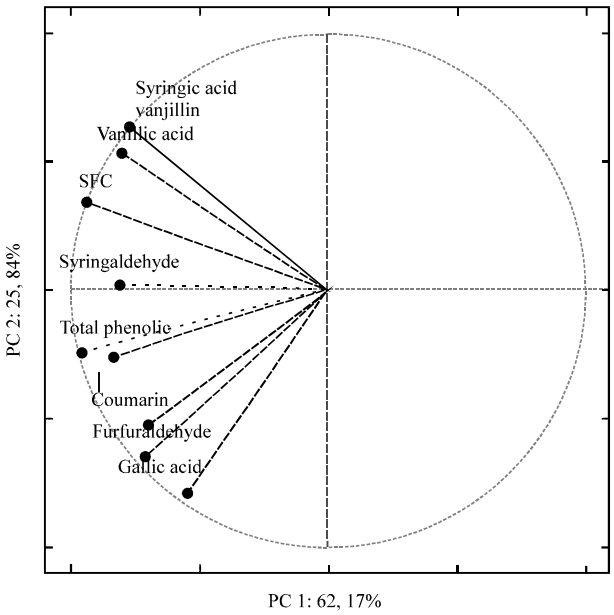

Fig. 5: Analysis of principal components of analytical data: Biplot with PC1 and PC2 showing a projection of the analysis on the plane defined by two principal components

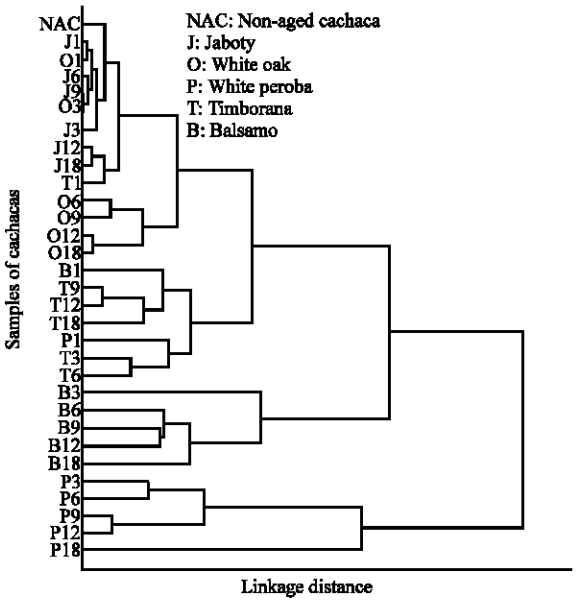

Fig. 6: Dendogram of all cachaças samples using unscaled data, complete linkage and Euclidean distances

CONCLUSIONS

The phenolic profile became more complex and the concentrations of every individual phenolic compound increased with more time aging of cachaças. The botanical origin of wood affected the phenolic composition of aged cachaças. Cachaça aged in white peroba presented a significant amount of vanillin then recommended this wood for use in the cachaça aging process because of the desirable sensory characteristics vanillin confers on alcoholic beverages. It is established that

vanillic acid and syringic acid, result from cinnamic aldehyde, vanillin and syringaldehyde, respectively. Cachaças aged in Brazilian woods contain larger amounts of coumarin than cachaça aged in white oak. None scopoletin was found in any cachaça. The phenolic composition and your concentration determinate the antioxidant capacity in the cachaça aged. The aged cachaças with the highest antioxidant capacity were balsamo, followed by white peroba, timborana, white oak and jaboty. The non-aged cachaça showed very low capacity antioxidant. A good correlation was observed between the total phenolic and I analyses and total phenolic and SPC. However, moderate correlation was observed between SPC and I. In general, it was found that, starting from the 3rd month of aging; it was possible to differentiate and classify the most aged cachaça into groups. This work suggests that 2-furfuraldehyde can degrade in cachaça aged in timborana, white peroba and balsamo, in accordance with other works that indicate that the furfuraldehyde is reducedto the corresponding alcohol by biological mechanisms or chemical reduction. The objective of this work is not to recommend the consumption of cachaça or other alcoholic beverages as a source of antioxidants. However, for people who already consume these beverages in moderation, the consumption of aged cachaça must offer some nutraceutical advantage in comparison to non-aged cachaça due to the consumption of exogenous antioxidants. Further epidemiological studies are needed to clarify how antioxidants in alcoholic beverages are related positively to human health. The casks made from Brazilian woods balsam, followed by white peroba and timborana have higher antioxidant capacity and phenolic profile more complex and quantitatively greater than the white oak. Therefore, these Brazilian wood casks already used by Brazilian cooperage industry are more indicated for the replacement of white oak, considering the variable here studied.

ACKNOWLEDGMENTS

The authors thank CNPq, PRONEX/CNPq/FAPEAL (Brazil) for their financial support of this study.

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