Formation of volatile and maturation-related congeners during the aging of sugarcane spirit in oak barrels

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The aging process of distilled spirits is a complex system based on the extraction of molecules from the wood and interactions with the liquid, the phenomenon of migration of wood constituents, as well as the formation and degradation of several compounds. Volatile and maturation-related congeners were evaluated during the aging process of *cachaça*, a Brazilian sugarcane spirit aged in oak barrels. Aged *cachaça* presented alterations in the levels of ethanol, higher alcohols, acetaldehyde, volatile acidity, ethyl acetate, total volatile congeners, isoamyl alcohol, ethyl carbamate and copper. The aging markers (gallic acid, furfural, 5-hydroxymethylfurfural, vanillic acid, syringic acid, vanillin, syringaldehyde, sinapaldehyde and coniferaldehyde) were compared with the compounds found in whisky, cognac, armagnac, bourbon and brandy. Monitoring the generation and evolution of congeners during the aging process allowed the characterization of *cachaça* and the identification of product maturity. Copyright © 2014 The Institute of Brewing & Distilling

Keywords: sugarcane spirit; volatile congeners; maturation compounds; aging; oak

Introduction

Cachaça is the typical and exclusive sugarcane spirit of Brazil. It is the fourth most produced distilled beverage in the world. It is produced by the distillation of fermented sugarcane juice, reaching 38–48% ethanol by volume at 20 °C. Sugarcane spirit production involves the extraction of sugarcane juice, fermentation and distillation. After these processes, aging is of fundamental importance, although not mandatory for *cachaça*. Nevertheless, Brazilian law establishes that aged *cachaça* is the spirit matured in wooden casks (maximum capacity of 700 L) for a period of not less than one year *(1)*.

The distillation of a sugarcane derived fermented wort results in a product that is basically composed of water, alcohols, aldehydes, acids, ketones and esters. The recently distilled beverage presents aggressive sensory features and a strong alcoholic flavour, aversive attributes that can be attenuated by aging.

The volatile fraction of sugarcane spirits plays an important role in ensuring product quality and acceptance. Certain compounds may enhance the quality of *cachaça* and add aromatic notes to the bouquet of the beverage; however, above determined limits, they may characterize flaws. Volatile congeners are formed during the fermentation process and their presence in the product depends on the distillation method used.

Several compounds are considered contaminants in *cachaça*, such as methanol, ethyl carbamate, acrolein, sec-butyl alcohol, *n*-butyl alcohol and copper. Since ethyl carbamate is a potentially carcinogenic compound normally present in *cachaça* *(2)*, Brazilian law establishes 150 µg/L as its highest limit in this beverage *(1)*.

The aging process of distilled spirits is a complex system based on the extraction of molecules from the wood and interactions with the beverage. Maturation is the major factor in the characterization of distilled spirits, because approximately 60% of the aromatic compounds present in the product come from the wood or the interaction of wood and distillate components *(3)*. Numerous physicochemical interactions occur between the wood and the spirit during aging, and several phenomena of migration of non-volatile and volatile compounds of wood to spirit take place. The evolution of phenolic compounds, beverage oxidation and the stabilization of colour and flavour, as well as the development of notes of wood, contribute to the richness and complexity of the distilled beverage's flavour and consequently result in a value-added product *(4)*.

Wood is a complex biological system composed mainly of cellulose, hemicelluloses and lignin. These different compounds do not behave in the same way when interacting with a distilled spirit, favouring specific reactions of the system wood–beverage. Cellulose, the major component of wood, is not normally degraded by the thermal treatment of wood during the barrel-making process. Hemicelluloses (pentosans) can generate pentoses by hydrolysis and, posteriorly, furfural, an aromatic molecule identified in aged spirits *(3)*. Lignin generates two different groups of compounds, one composed of coniferaldehyde, vanillin and vanillic acid, originating from the guaiacyl structure, and the other composed of sinapaldehyde, syringaldehyde and syringic acid, originating from the syringyl structure *(5)*.

Two possible pathways have been proposed for the mechanisms involved in the extraction of maturation-related congeners from lignin during the aging process. One of them is the simple

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extraction of phenolic compounds present in wood, which are incorporated into the beverage. The other involves the extraction of lignin from wood owing to the action of ethanol, forming an ethanol–lignin compound, which is subsequently degraded to simple phenolic compounds. The oxidation of sinapaldehyde originates syringaldehyde, which, in turn, can be oxidized to form syringic acid. The oxidation of coniferaldehyde forms vanillin, which can be oxidized to form vanillic acid (6).

Taking into consideration the pathways of formation of maturation-related congeners in aged distilled spirits, the analytical monitoring of the formation of each congener during the aging process is of vital importance. Therefore, the aim of this study was to evaluate the volatile congeners and the development of maturation-related congeners during the aging process of *cachaça* in oak barrels.

Materials and methods

The sugarcane spirit used in this study was produced in 2010 in the distillery of the Agro-Food Industry and Nutrition Department of the College of Agriculture 'Luiz de Queiroz' of the University of São Paulo. The wort was prepared using sugarcane variety SP 81-3250. The sugarcane juice was extracted using a stainless steel presser and it underwent a thermal treatment (105 °C) to eliminate contaminating microorganisms and a decantation for 2 h for colloidal precipitation. Fermentation was performed in 200 L tanks using *Saccharomyces cerevisiae* strain CA-11 (LNF Latino Americana, Bento Gonçalves, RS, Brazil) at 30 °C for 24 h and distillation was carried out in a 150 L copper pot still.

In the present work, double distillation was applied, based on the methodology used for whisky production (7). In the first distillation, all of the alcohol was extracted from the wine, until the distillate in the condenser outlet presented approximately 3% ethanol (v/v), measured using an alcohol meter, resulting in a distillate fraction named low wines. It was necessary to apply the first distillation process to three batches of wine to produce an adequate volume of low wines in order to perform the second distillation. The low wines underwent the second distillation process (carried out in triplicate). In the second distillation, the 'head' (1.5% of the boiler useful volume), the 'heart' (distillate fraction recovered after the 'head' fraction and until the distillate fraction in the condenser outlet presented 60% ethanol v/v) and the 'tail' (distillate fraction recovered after the 'heart' fraction and until the distillate fraction in the condenser outlet presented 3% ethanol v/v) distillate fractions were separated. The 'heart' distillate fraction characterized the double-distilled sugarcane spirit. (8).

The barrels (50 L) for aging the spirit were made from oak wood (*Quercus sessile*). The barrels were not toasted or charred. The staves were thermal treated only for bending.

The double-distilled sugarcane spirit (approximately 200 L at 68.5% ethanol v/v) was aged in the barrels for 180 days (in triplicate) at room temperature (22 ± 5 °C) and a relative humidity of 55 ± 10%, and protected from vibrations. The remaining volume of the sugarcane spirit was stored in a 50 L glass flask and used as the control. Samples of 25 mL were collected at the same level from the centre of the barrels every 10 days for the analysis of maturation-related congeners and every 20 days for the analysis of volatile compounds. The sugarcane spirit was analysed to quantify ethanol and copper according to official methods (1).

The content of acetaldehyde, ethyl acetate, methanol, higher alcohols (*n*-propyl, isobutyl and isoamyl), 1-propyl and 2-butyl alcohols, and acetic acid was determined using gas chromatography.

Standards used in this study were all from Merck (Darmstadt, Germany), purity >99%. Samples were spiked with the internal standard (4-methyl-2-pentanol). Aliquots of 1.0 μL were automatically injected into a gas chromatographic system (Shimadzu, QP-2010 PLUS, Tokyo, Japan) equipped with a Stabilwax-DA column (crossbond carbowax polyethylene glycol, 30 m × 0.18 mm × 0.18 μm film thickness) and a flame ionization detector. The analyses were performed at a 1:25 split ratio, in triplicate. Nitrogen was used as the carrier gas (flow rate of 1.5 mL/min, total flow of 42 mL/min and pressure of 252.3 kPa). The temperatures of both the injector and the detector were set at 250 °C. The oven temperature programme was 40 °C for 4 min, followed by an increase to 120 °C at 20 °C/min, kept for 1 min, and then to 180 °C at 30 °C/min, and maintained for 4 min (9).

The samples were analysed for ethyl carbamate in a gas chromatograph coupled to a mass spectrometer, model GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan), using electron impact ionization with an energy of 70 eV and a chromatography capillary column with polar phase (esterified polyethylene glycol, HP-FFAP; 50 m × 0.20 mm × 0.33 μm stationary phase film thickness). The temperatures of the injector and the detector interface were 230 and 220 °C, respectively. The following temperature programme was used in the oven: starting with 90 °C for 1 min; increasing to 150 °C at a rate of 10 °C/min; followed by heating up to 230 °C at a rate of 30 °C/min; and maintained at this temperature for 2 min. An aliquot of 1.0 μL was injected using the splitless injection mode, in duplicate. Helium was used as the carrier gas (flow rate of 1.2 mL/min). Selected ion monitoring acquisition was employed, monitoring the *m/z* 62 ions for ethyl carbamate and *m/z* 75 ions for methyl carbamate, used as the internal standard (10). Quantification was performed comparing the chromatographic results of the samples with an analytical curve obtained using an ethyl carbamate stock solution. All reagents were analytical grade. Ethyl carbamate (99%) and methyl carbamate (98%) were purchased from Sigma-Aldrich (St Louis, MO, USA). For the dilutions, ethanol gradient grade for gas chromatography (Merck, Darmstadt, Germany) and ultrapure water (Milli-Q) were used.

The aging congeners were analysed using high-performance liquid chromatography (HPLC) in a Shimadzu equipment, model LC-10 AD (Tokyo, Japan), with two Shimadzu LC-20 AD pumps, a UV–vis detector Shimadzu SPD-20A, a system controller CBM-20A and an automated injection system (20 μL) with gradient elution. The standards used in this study were gallic acid, 5-hydroxymethylfurfural, furfural, vanillin, vanillic acid, syringaldehyde, sinapaldehyde, syringic acid and coniferaldehyde, all from Sigma-Aldrich (St Louis, MO, USA), purity >99%. The HPLC method had two mobile phases composed of water–acetic acid (98:2, v/v) and methanol–water–acetic acid (70:28:2, v/v/v) at a flow rate of 1.25 mL/min. A pre-column, Shimadzu VP-ODS (1 cm × 4.6 μm), and a C_{18} reversed-phase column model Shimpack VP-ODS (4.6 mm, 25 cm × 5 μm) thermostabilized at 40 °C were used. The UV detector was programmed to operate at variable wavelengths, according to Bortoletto and Alcarde (9). The samples were previously filtered using a Millex-HV filter with PVDF membrane (diameter 13 mm, pore size 0.45 μm).

Results and discussion

The compounds analysed using chromatographic methods were quantified above the detection limit, except for sec-butyl alcohol. The chromatographic parameters retention time, detection limit

and quantification limit of volatile and maturation-related compounds, as well as the correlation coefficients (a, b, r^2) of the calibration curves of the chromatographic analysis are shown in Table 1 (11).

The concentration of volatile congeners gradually increased during the aging period of *cachaça*. The sugarcane spirit stored in a glass flask (control) also presented increased concentration of volatile congeners, but not as much as the *cachaça* aged in the oak barrels (Fig. 1).

The concentration of ethanol decreased by 4.4% on average in the *cachaça* aged in oak barrels, reaching 65.5% at the end of the 180 day period of aging (Fig. 1A). This decrease occurred owing to the preferential evaporation of ethanol in relation to water because of the high initial ethanol content (68.5% v/v). However, since an increase in acetaldehyde was observed, ethanol also underwent oxidation to ethanal. During the aging period of 180 days, the content of acetaldehyde (Fig. 1C) and ethyl acetate (Fig. 1E) increased by 79.3 and 107.3%, respectively, in the *cachaça* aged in oak barrels, and by 30.2 and 37.8%, respectively, in the control.

The typical, agreeable, pungent and mild taste and flavour imparted by aging to *cachaça* are mainly attributed to the formation of aromatic esters, compounds that contribute to the formation of the bouquet. The esters are produced during fermentation by yeast, as well as during the aging process by the esterification of fatty acids with ethanol. Ethyl acetate, the major component of this group, is responsible for the agreeable flavour of aged spirits (12). These reactions also take place in beverages stored in inert containers, but they are slower owing to a lack of specific reactions that accelerate the oxidation process of the distilled spirits during aging.

The principal higher alcohols produced by yeast in sugarcane juice wort are *n*-propyl, isobutyl and isoamyl (Fig. 1G) alcohols, which increased by 25.7, 18.4 and 16.1%, respectively, during aging. Therefore, the total concentration of higher alcohols (380.26 mg/100 mL anhydrous ethanol – AE) in aged *cachaça*, including *n*-propyl, isobutyl and isoamyl alcohols (1), increased by 18.5% during the period of 180 days of aging (Fig. 1B), exceeding the maximum amount permitted by Brazilian law (360 mg/100 mL AE) (1). In the control, *n*-propyl, isobutyl, isoamyl and total higher alcohols increased by 15.6, 8.9, 4.3 and 7.5%, respectively.

The concentration of acetic acid increased by 115.6% in aged *cachaça* (Fig. 1D), but even with this large increase, the beverage presented volatile acidity well below the maximum amount permitted by Brazilian law (150 mg/100 mL AE expressed in acetic acid) (1). The concentration of acetic acid in the control increased by just 8.5%. Oxidation of ethanol to acetaldehyde also promotes the formation of acetic acid. Furthermore, acetic acid and methanol are also formed by the degradation of hemicellulose (12). Aging distilled spirits in wooden barrels imparts nonvolatile organic acids to the beverage, increasing its acidity.

Methanol concentration increased by 22.9% in aged *cachaça*. The presence of methanol in distilled spirits is undesirable because of its toxicity even at low concentrations. Although the aging process increases the amount of methanol by oxidation, the origin of this alcohol is normally associated with the degradation of pectin, a polysaccharide found in sugarcane. The sugarcane spirits analysed in this study presented methanol concentrations ranging from 3.0 to 3.8 mg/100 mL AE, well below the maximum amount permitted by Brazilian law (20 mg/100 mL AE) (1).

Table 1. Retention time (RT), detection limit (DL) and quantification limit (QL) of volatile and maturation-related congeners and correlation coefficients (a, b, r^2) of the calibration curves for *cachaça* aged for 180 days in oak barrels

| Compound	RT (min)	DL (mg/100 mL AE)	QL (mg/100 mL AE)	a	b	r^2
Volatile congeners						
Acetaldehyde	1.59	0.081	0.251	0.8094	−0.0648	0.998
Ethyl acetate	2.71	0.043	0.132	0.0370	0.0913	0.995
n-Propyl alcohol	5.74	0.056	0.186	0.2319	0.0094	0.999
Isobutyl alcohol	6.52	0.026	0.094	0.0204	0.0031	0.999
Isoamyl alcohol	8.02	0.017	0.041	0.1763	0.0151	0.999
Acetic acid	10.47	0.490	1.721	0.6238	0.1111	0.996
Contaminant congeners						
Methanol	2.92	0.161	0.544	0.7848	0.0483	0.965
n-Butyl alcohol	7.31	0.061	0.200	0.2036	0.1331	0.997
sec-Butyl alcohol	5.34	0.215	0.710	0.2667	0.0024	0.999
Ethyl carbamate[a]	10.12	0.230	0.710	36.1655	829.014	0.998
Maturation congeners						
Gallic acid	6.36	0.031	0.096	1823.23	56.16	0.992
5-Hydroxymethylfurfural	11.90	0.017	0.046	6074.21	90.48	0.997
Furfural	14.10	0.010	0.046	5778.61	1034.18	0.997
Vanillic acid	24.02	0.048	0.158	1262.14	256.66	0.998
Syringic acid	26.61	0.029	0.096	2427.27	−103.23	0.998
Vanillin	27.09	0.026	0.067	3111.94	−86.94	0.999
Syringaldehyde	29.17	0.054	0.166	1078.18	340.49	0.996
Coniferaldehyde	34.81	0.022	0.066	4549.27	147.16	0.998
Synapaldehyde	35.87	0.030	0.094	3216.12	101.88	0.995

[a]μg/L.
AE, Anhydrous ethanol.
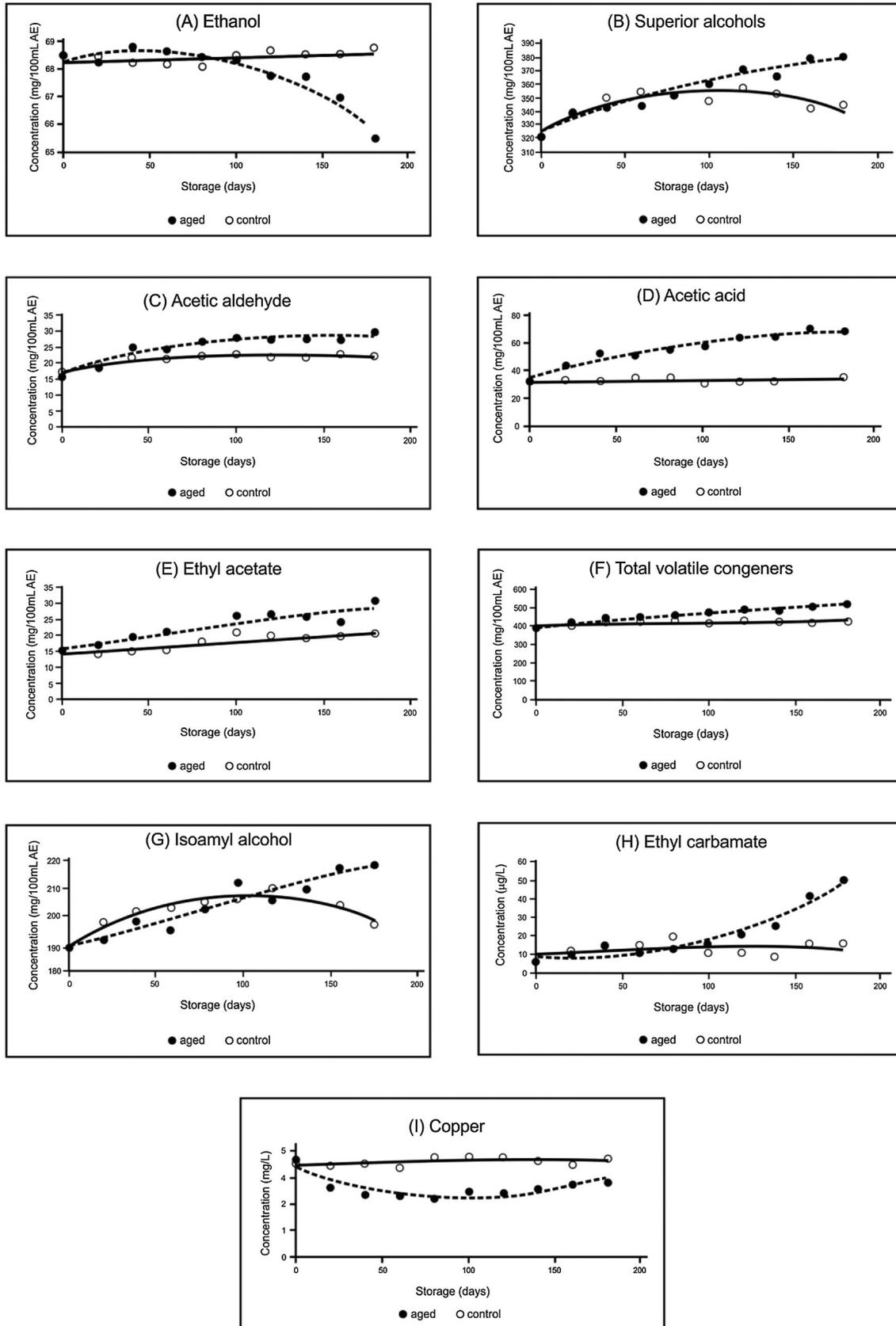
Figure 1. Concentration of some volatile congeners, ethyl carbamate and copper in *cachaça* during the aging period of 180 days in oak barrels. Dots and circles include the error bars.

Brazilian law establishes a maximum amount of 5 mg/100 mL AE for the sum of the concentrations of furfural and hydroxymethylfurfural in sugarcane spirits (1). In the control, the sum of these compounds was 0.76 mg/100 mL AE, whereas at the end of the aging period, it was 6.17 mg/100 mL AE in aged *cachaça* (Table 2), exceeding the maximum amount permitted by Brazilian law.

The coefficient of congeners in *cachaça* is the sum of aldehydes, esters, higher alcohols, volatile acidity, furfural and hydroxymethylfurfural (1). In the present study, the concentration of these compounds increased during the aging process, leading to an increase in the coefficient of congeners by 34.3% in aged *cachaça* (Fig. 1 F), whereas in the control it increased by just 9.9%.

The concentration of *n*-butyl alcohol increased by 51% in both the aged *cachaça* and the control. Nonetheless, the values found (0.8–1.1 mg/100 mL AE) were below the maximum amount permitted by Brazilian law (3 mg/100 mL AE) (1). The concentration of sec-butyl alcohol in both the aged *cachaça* and the control was below detection limit.

The level of copper in *cachaça* is due to extraction of copper from the distiller during the distillation process. The concentration of copper decreased by 23.1% in aged *cachaça* at the end of the aging period, whereas in the control the initial concentration was maintained throughout this period (Fig. 1). Phenolic compounds may react with the copper present in distilled spirits, which causes a darkening of the beverage. Additionally, copper may be absorbed or adsorbed by the wood of the barrel (13).

Catão *et al.* (14) reported an increase in the concentrations of volatile acidity, esters, aldehydes, furfural and higher alcohols in sugarcane spirits aged in wooden barrels. The authors also affirmed that the concentrations of ethanol and methanol did not change and that the copper content decreased slightly during the aging period. Parazzi *et al.* (15) analysed the chemical composition of sugarcane spirits aged for 3 years in oak barrels. The concentrations of ethanol, acetaldehyde, ethyl acetate, methyl alcohol, acetic acid, *n*-propyl, isobutyl and isoamyl alcohols increased, whereas the concentration of *n*-butyl alcohol decreased.

In this study, ethyl carbamate was formed during the aging period. The concentration of this compound tripled in the control and stabilized from day 40 onwards. In aged *cachaça*, a significant increase in this parameter was observed, reaching a 10-fold higher concentration at the end of the 180 days.

Despite the fact that most of the ethyl carbamate forms 24–48 h after distillation (16), the final ethyl carbamate concentration in distilled spirits depends on the initial level of the compound measured after distillation plus the ethyl carbamate formed from active precursors, such as hydrogen cyanide, cyanate and copper cyanide complexes. The components extracted from oak are involved in the conversion of cyanide to cyanate by the action of peroxides. Ethyl carbamate concentrations rapidly increased over the 42 days of whisky maturation and plateaued at concentrations around 70 ppb until the end of the 3 years (17).

In *cachaça* that is not aged, the maximum concentration of ethyl carbamate is reached by the seventh day after distillation (18). Matured *cachaça* tends to be more contaminated with ethyl carbamate than fresh distilled *cachaça* (19). Anjos *et al.* (20) observed a significant increase in the concentration of ethyl carbamate in *cachaça* after a 12-month aging period in oak barrel. The formation of ethyl carbamate during the period of storage of *cachaça* is believed to take place gradually, as a result of reactions between ethanol and the urea formed during the degradation of nitrogen precursors, such as the amino acids arginine, ornithine and citrulline. Furthermore, carbamyl phosphate and the cyanide ion are mentioned as possible precursors in the formation of ethyl carbamate after the distillation process (21,22).

It should be noted that, within the frame of the present experiment, the aged spirit did not meet the Brazilian law quality criteria for furfural + HMF 5-hydroxymethylfurfural and higher alcohols. Nevertheless Brazilian law refers primarily to the non-aged spirit, namely 'white' *cachaça*. Aged *cachaça* should be related to aged spirits, such as whiskies and brandies (Tables 3 and 4), for which oak aging is an essential step towards their final quality.

In this study, all of the maturation-related congeners were found in aged *cachaça* in concentrations above the detection limit after day 10 of aging. Over 50% of the total concentration of congeners found in *cachaça* after 180 days of aging had been formed in the first 10 days of contact with the wood of the barrel, which indicates accelerated extraction and formation of compounds (Table 2).

Fig. 2 shows the concentration of each compound at the beginning (10 days) and end (180 days) of the aging period. The compounds that present the highest concentrations at day 10 are 5-hydroxymethylfurfural, syringaldehyde and furfural, and at day 180 they are syringaldehyde, 5-hydroxymethylfurfural and gallic acid. During the aging period, the participation of each compound changed, and decreased concentrations of coniferaldehyde and sinapaldehyde and increased concentrations of vanillic acid, syringic acid and vanillin were observed.

Gallic acid originates from wood tannins and it is one of the maturation-related congeners responsible for the viscosity and texture of the beverage developed during the aging process. This compound is extracted directly from wood and it is a non-volatile substance, that is, it does not participate in the aromatic bouquet of distilled spirits, but it contributes to enhance the quality of the beverage related to the visual and texture attributes (3). Although this compound was extracted from the beginning of the aging process, the rate of extraction was intensified from day 90 onwards, and its concentration had doubled at the end of the 180-day period.

Furfural was the only pre-existent maturation-related congener in the control. However, it reached a 20-fold higher concentration at the end of the 180 days (18.71 mg/L). Furfural and 5-hydroxymethylfurfural can be present in burned sugarcane, or they can be generated during the distillation process by pyrogenation of the organic matter precipitated at the bottom of the pot still. Moreover, Maillard reactions and caramelization caused by the thermal treatment of wood during the barrel-making process can also generate these compounds by the thermal breakdown of pentoses and hexoses, and during the aging process they are extracted and incorporated into the beverage (23).

According to Parazzi *et al.* (15), the content of both furfural and 5-hydroxymethylfurfural increase with aging. These are important molecules in the sensory evaluation of *cachaça*, since they are responsible for the peculiar colour of aged distilled spirits and actively participate in the flavour, generating agreeable aromatic notes of burned wood and almonds. Nevertheless, if present in high concentrations, they may be deemed a flaw, making the beverage disagreeable. In the present study, 65% of the final concentration of these compounds in aged *cachaça* was generated during the first 10 days of contact with wood; therefore, their extraction is simple and fast.

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Table 2. Concentration and standard deviation of maturation-related congeners in *cachaça* aged for 180 days in oak barrels

Aging period (days)	Maturation-related congener (mg/L)									
	GA	5-HMF	FR	CN	VN	VA	SP	SG	SA	Total
0	—	—	0.85 ± 0.05	—	—	—	—	—	—	0.85 ± 0.05
10	7.92 ± 1.39	15.12 ± 2.02	11.41 ± 0.06	2.23 ± 0.03	2.24 ± 0.29	2.12 ± 0.24	7.89 ± 0.27	11.01 ± 1.39	4.36 ± 0.93	64.29 ± 6.60
20	9.03 ± 1.30	15.23 ± 1.31	12.25 ± 0.61	2.61 ± 0.28	2.71 ± 0.36	1.79 ± 0.58	8.93 ± 0.34	15.06 ± 0.98	4.76 ± 0.59	72.38 ± 6.24
30	4.79 ± 0.55	15.17 ± 0.96	12.40 ± 0.89	2.96 ± 0.11	2.88 ± 0.25	1.68 ± 0.17	9.92 ± 0.40	10.57 ± 0.77	5.76 ± 0.45	66.13 ± 4.53
40	5.13 ± 0.57	15.93 ± 2.27	12.87 ± 0.49	3.22 ± 0.09	3.41 ± 0.17	2.00 ± 0.22	10.84 ± 0.25	11.55 ± 0.98	6.14 ± 0.55	71.09 ± 5.58
50	11.46 ± 0.58	15.69 ± 1.50	12.69 ± 0.54	3.25 ± 0.27	2.80 ± 1.03	2.61 ± 0.22	11.45 ± 0.44	18.23 ± 0.47	6.54 ± 2.50	82.73 ± 7.54
60	11.44 ± 0.43	16.55 ± 1.80	13.74 ± 0.32	3.30 ± 0.09	2.99 ± 0.83	3.16 ± 0.45	11.66 ± 0.54	19.05 ± 0.37	6.80 ± 0.03	88.68 ± 4.86
70	11.42 ± 1.99	17.00 ± 1.07	13.22 ± 1.60	3.75 ± 0.02	3.95 ± 0.53	2.52 ± 1.06	12.35 ± 0.16	19.68 ± 1.09	6.29 ± 1.16	90.18 ± 8.66
80	10.41 ± 2.08	17.07 ± 1.28	14.02 ± 0.49	3.64 ± 0.11	4.56 ± 0.04	2.57 ± 0.63	12.49 ± 0.30	20.95 ± 0.58	7.90 ± 0.38	93.62 ± 5.88
90	10.80 ± 1.45	17.45 ± 1.24	14.32 ± 0.78	3.75 ± 0.26	5.20 ± 0.18	2.48 ± 0.26	12.58 ± 0.56	22.19 ± 0.21	8.19 ± 0.59	96.95 ± 5.53
100	11.63 ± 0.87	17.33 ± 0.61	14.66 ± 0.79	0.26 ± 0.05	5.29 ± 0.09	2.65 ± 0.01	12.50 ± 0.51	18.00 ± 0.75	8.97 ± 0.15	89.29 ± 3.84
110	13.09 ± 2.02	18.02 ± 1.61	15.09 ± 0.77	0.33 ± 0.08	5.64 ± 0.31	2.62 ± 1.41	12.80 ± 0.35	20.10 ± 1.09	9.74 ± 0.66	97.44 ± 8.30
120	17.33 ± 1.59	18.64 ± 1.59	15.30 ± 0.88	0.21 ± 0.12	5.77 ± 0.14	4.20 ± 0.04	13.04 ± 1.12	20.18 ± 0.42	10.07 ± 0.84	104.74 ± 6.73
130	18.60 ± 1.44	19.38 ± 1.55	15.75 ± 0.74	0.29 ± 0.05	6.17 ± 0.42	4.75 ± 0.51	13.30 ± 0.97	21.26 ± 0.08	10.86 ± 0.73	110.36 ± 6.49
140	23.23 ± 0.74	19.68 ± 1.66	16.96 ± 0.35	0.32 ± 0.09	6.99 ± 0.04	4.65 ± 1.02	13.84 ± 0.29	24.38 ± 1.77	11.58 ± 0.77	121.62 ± 6.72
150	22.29 ± 2.52	19.95 ± 1.31	16.43 ± 1.20	0.32 ± 0.09	7.28 ± 0.42	4.74 ± 0.59	12.84 ± 0.43	28.05 ± 2.25	11.69 ± 0.69	123.61 ± 9.48
160	22.00 ± 1.77	20.93 ± 1.28	17.41 ± 1.67	0.59 ± 0.19	7.70 ± 0.16	5.38 ± 0.42	4.37 ± 1.86	26.00 ± 1.02	12.89 ± 0.67	117.27 ± 9.03
170	21.13 ± 2.48	21.26 ± 1.53	17.53 ± 1.22	0.65 ± 0.09	7.95 ± 0.17	5.76 ± 0.32	2.24 ± 0.19	27.00 ± 1.65	13.37 ± 0.65	116.90 ± 8.29
180	22.78 ± 1.38	22.29 ± 1.90	18.71 ± 1.13	0.82 ± 0.23	8.41 ± 0.07	5.99 ± 0.70	2.61 ± 0.25	28.95 ± 2.24	14.05 ± 1.25	124.73 ± 9.14
ER	2.87	1.47	1.63	—	3.77	2.82	—	2.6	3.22	—

GA, gallic acid; 5-HMF, 5-hydroxymethylfurfural; FR, furfural; CN, coniferaldehyde; VN, vanillin; VA, vanillic acid; SP, synapaldehyde; SG, syringaldehyde; SA, syringic acid; ER, evolution rate.

Table 3. Ratio between maturation-related congeners in aged distilled beverages

Ratio[a]	Cachaça[b]	Armagnac[c]	Whisky[d]	Bourbon[e]	Brandy[f]
SG/SP	11.1			6.5	
SA/SG	0.5				
VN/CN	10.3			2.3	
VA/VN	0.7	1.0	0.6		0.4
SG/VN	3.4	1.4–2.5	1.7–2.5	1.7	2.8
SA/VA	2.4	1.5			2.3
GA/VN	2.7	0.1–0.3			1.7

[a]SG, syringaldehyde; SP, synapaldehyde; SA, syringic acid; VN, vanillin; CN, coniferaldehyde; VA, vanillic acid; GA, gallic acid.
[b]Present study.
[c]Puech (26).
[d]Aylott and Mackenzie (5).
[e]MacNamara et al. (27).
[f]van Jaarsveld et al. (28).

Table 4. Average composition (mg/L) of maturation-related congeners in aged distilled beverages

Maturation-related congener	Cachaça[a]	Scotch whisky[b]	Cognac[c]
Gallic acid	22.8	—	22.0
Furfural	22.3	16.5	—
Vanillin	8.4	2.8	5.8
Vanillic acid	6.0	1.8	3.1
Syringaldehyde	29.0	5.1	10.9
Syringic acid	14.1	2.5	4.0

[a]Present study.
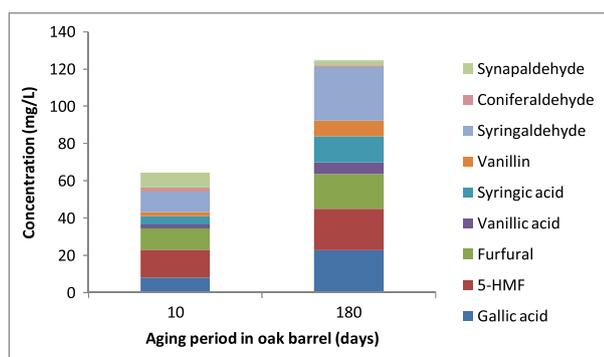[b]Aylott and Mackenzie (5).
[c]Viriot et al. (29).

Figure 2. Concentration of maturation-related congeners in aged *cachaça* at the beginning (10 days) and at the end (180 days) of the aging period. Compounds are listed in order given in the bars. This figure is available in colour online at wileyonlinelibrary.com/journal/brewing.

The kinetics of congeners derived from lignin varied during the period of contact of *cachaça* with the wooden barrel. The content of vanillic acid, syringic acid, vanillin and syringaldehyde gradually increased until the end of the experiment. The content

of coniferaldehyde increased until day 90, but decreased by 78% at the final sampling date. Sinapaldehyde presented similar characteristics, with increased content until day 150, and decreasing by 80% at day 180. Despite the drastic decrease in coniferaldehyde and sinapaldehyde concentrations in the final product, the total sum of maturation-related congeners increased during the aging period.

The most distinctive compound at the end of the 180-day aging period was syringaldehyde. Bortoletto and Alcarde (9) studied the chemical profiles of sugarcane spirits aged in casks made from different Brazilian woods and compared them with those obtained in oak casks. The authors observed that syringaldehyde was the predominant compound in *cachaça* aged in oak barrels, but it was not high in *cachaça* aged in barrels made from other types of wood.

The evolution rate (ER) of the compounds that presented an increase in concentration during the aging period was analysed by comparing the values found at days 10 and 180 (Table 2). The ER of vanillin was 3.7, which indicates this is a compound that, in addition to being extracted directly from wood, is also generated by reactions that occur during the aging process. The ER of vanillic acid was 2.8, and its concentration increased from day 120 onwards. The ERs of syringaldehyde, syringic acid and gallic acid were 2.6, 3.2, and 2.9, respectively. The ERs of furfural and 5-hydroxymethylfurfural were lower – 1.6 and 1.4, respectively – which characterizes compounds easily extracted from wood and not predominantly originating from complementary reactions.

Anjos et al. (24) analysed the concentration of phenolic compounds in *cachaça* during the aging process in a 200 L oak barrel and found the following ER values: gallic acid, 3.83; vanillin, 3.70; vanillic acid, 3.02; syringadehyde, 3.69; and syringic acid, 4.09.

The aging of sugarcane spirits in wooden barrels leads to a progressive increase in the concentrations of phenolic acids and aldehydes, following the sequence: cinnamaldehydes (coniferaldehyde and sinapaldehyde), benzaldehydes (vanillin and syringaldehyde) and benzoic acids (vanillic and syringic acids) (25).

As observed by Anjos et al. (24), the present study also showed significant positive linear correlations between vanillin and vanillic acid (0.8122) and syringaldehyde and syringic acid (0.7941). The changes that occur in the concentrations of vanillic and syringic acids during the aging process of sugarcane spirits in wooden barrels might be related to the oxidation of vanillin and syringaldehyde, respectively. Therefore, in addition to the ethanolic extraction from wood, vanillic and syringic acids may be formed by the oxidation of vanillin and syringaldehyde, respectively, during the aging process.

Based on two different formation pathways of maturation-related congeners, the guaiacyl (coniferaldehyde, vanillin and vanillic acid) and syringyl (sinapaldehyde, syringaldehyde and syringic acid) pathways, it is possible to establish relationships between compounds of the same pathway (Table 3). Both pathways presented similar values for the ratio between the intermediary compound and the precedent compound (syringaldehyde/synapaldehyde and vanillin/coniferaldehyde), as well as for the ratio between the final compound and the intermediary compound (syringic acid/syringaldehyde and vanillic acid/vanillin) in this study. The ratios allow the analysis of the balance between the concentrations of maturation-related congeners in both pathways and, consequently, the evaluation of the chemical complexity of a distilled spirit during the aging process. This evaluation showed uniformity between the formation of congeners in both pathways, which favours the chemical and sensorial quality of the beverage.

Some authors have also cited ratios of hemicellulose-derived aromatic compounds (Table 3) for Armagnac brandy *(26)*, bourbon whisky *(27)*, brandies *(28)* and Scotch whisky *(5)*. Dias *et al. (25)* affirmed that the concentration of phenolic compounds associated with oxidation during aging depends on a number of factors, such as the type of wood (permeability, porosity, density), the size and geometry of the barrels, and storage conditions.

Anjos *et al. (24)* reported a progressive increase in the concentration of phenolic compounds in *cachaça* aged for 12 months in a 200 L oak barrel. The main compounds incorporated into the spirit were syringaldehyde and gallic acid. Dias *et al. (25)* observed the prevalence of ellagic acid and vanillic acid in *cachaça* stored in oak barrels for 6 months.

The chemical quality of aged distilled spirits depends on the balance of the concentrations of their components to result in a beverage that presents aromatic complexity and intensity. In the present study, a balance between both pathways of formation of congeners derived from lignin, showing the stability and maturity of aged *cachaça*, was observed. The composition of maturation-related congeners found in the *cachaça* obtained in this study was similar to the average profile of phenolic compounds found in whiskies aged for 12 years and cognacs aged for 10 years (Table 4).

The concentration of maturation-related congeners in *cachaça* aged in oak barrels changed during the aging period according to the characteristic of each compound related to the pathway of its formation from the respective macromolecule of origin in the wood.

The study of the kinetics of formation of maturation-related congeners is important to understand the aging process of distilled spirits. Monitoring the evolution and generation of maturation-related congeners during the aging process can help characterize the beverage and identify its maturity.

Acknowledgements

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