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Certification of amburana in the aging of cachaça

Certificação da amburana no envelhecimento da cachaça

Certificación amburan en el envejecimiento de cachaça

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Abstract

Cachaça is defined as sugarcane spirit produced in Brazil, but it still lacks references that valorize the aging in native woods. Amburana (*Amburana cearensis*) is a Brazilian tree widely used in making casks for storing cachaça. Among its phenolic derivatives, coumarin stands out. Its content is determinant in the sensorial characteristics developed in the beverage during the aging process. We propose a simple and rapid method (15 min) that allows the certification of the storage of cachaça in amburana barrels and the determination of the coumarin content. Using high-performance liquid chromatography, the identity of the wood is attested by the peculiarities of the chromatographic profile obtained with a UV detector (274 nm) in an isothermal environment (40 ^oC). Coumarin was easily identified among the peaks obtained, and it could be quantified using a standard curve.

Keywords: Amburana; Cachaça; Coumarin.

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Resumo

A cachaça é definida como a aguardente de cana produzida no Brasil, mas ainda carece de referências que valorizem o envelhecimento em madeiras nativas. Amburana (Amburana cearensis) é uma árvore brasileira amplamente utilizada na fabricação de tonéis para armazenamento de cachaça. Dentre seus derivados fenólicos, destaca-se a cumarina. Seu conteúdo é determinante nas características sensoriais desenvolvidas na bebida durante o processo de envelhecimento. Propomos um método simples e rápido (15 min) que permite a certificação do armazenamento da cachaça em barris de amburana e a determinação do teor de cumarina. Por meio da cromatografia líquida de alta eficiência, a identidade da madeira é atestada pelas peculiaridades do perfil cromatográfico obtido com detector de UV (274 nm) em ambiente isotérmico (40 ^{o}C). A cumarina foi facilmente identificada entre os picos obtidos, podendo ser quantificada por meio de uma curva padrão.

Palavras-chave: Amburana; Cachaça; Cumarina.

Resumen

La cachaça se define como aguardiente de caña de azúcar producido en Brasil, pero aún carece de referencias que valoricen la crianza en maderas nativas. Amburana (Amburana cearensis) es un árbol brasileño muy utilizado en la fabricación de toneles para almacenar cachaça. Entre sus derivados fenólicos destaca la cumarina. Su contenido es determinante en las características sensoriales que se desarrollan en la bebida durante el proceso de envejecimiento. Proponemos un método simple y rápido (15 min) que permite la certificación del almacenamiento de cachaça en barriles de amburana y la determinación del contenido de cumarina. Mediante cromatografía líquida de alta resolución, la identidad de la madera queda atestiguada por las peculiaridades del perfil cromatográfico obtenido con un detector UV (274 nm) en un ambiente isotérmico (40 ^{o}C). La cumarina se identificó fácilmente entre los picos obtenidos y se pudo cuantificar utilizando una curva estándar.

Palabras clave: Amburana; Cachaca; Cumarina.

1. Introduction

There are numerous phenolic derivatives (PD) in plants. The most abundant is lignin, a resinous macromolecule present in all parts of vegetables and corresponding to about 25% of the mass of the trunks and branches, to which it gives rigidity and resistance to mechanical and microbiological attacks. In much smaller proportions, several other classes of PD occur, with important and even essential functions in the context of reproduction, growth and defense against pathogens, parasites and predators. By giving color to flowers, for example, they act in the attraction of animals and insects, such as birds, butterflies and bees, which intervene in the pollination, process (Hoda et al., 2019, Grijalva et al., 2020). Among the PD, there are hundreds of structures of tannins, flavonoids and coumarins, antioxidants that, although not recognized as essential in human nutrition, are widely valued as herbal medicines.

The process of maturation (or aging) of the distillates occurs from the absorption of PD from the wood of the barrels in which they are stored. In very small proportions, on the order of milligrams per liter or less, these compounds confer important peculiarities of color, flavor and aroma. It is an empirical practice, dating back millennia in human history. From the middle of the 20th century, it began to be characterized in the context of chemical transformations and physiological effects (Matos et al., 2015; Silva et al., 2020).

White oak (*Quercus alb*a), a native tree in several countries of Europe and North America, is widely used in the aging of wines, whiskeys, cognacs, rums, tequilas and other beverages (GRANADOS et al., 2016). In Brazil, many producers import oak barrels (both new and used) for the maturation or aging of cachaça.

However, cachaça is conceptualized as a “distilled beverage produced exclusively in Brazilian territory”, and it is relevant to associate its identity with the appreciation of native flora. Amburana (*Amburana cearensis*) is one of the Brazilian trees traditionally used in making casks for storing cachaça. Despite the scientific name, which refers to the state of Ceará, it is known to occur in practically all Brazilian states, although with different names in each region: cumaru, baru, cabocla, cherry, emburana, imburana, or umburana (Braga, 2001). It stands out from the other types of wood because of its coumarin content, a compound with a sweet, characteristic and pleasant odor, similar to that of vanillin.

Coumarin (simple or aglycone) has the typical structure of hundreds of benzopyrones, referred to globally as coumarins, in which it is found free or in the form of glycosides. They are PD that occur in countless plants consumed in human and animal foods, including seeds, roots, stems, flowers and fruits. In addition to applications in food, beverages, perfumes and cosmetics, coumarins are highly valued in medicinal chemistry (Matos et al., 2015; Kirsch et al., 2016). Amburana is a tree suitable for enhancing the “Brazilianness” of cachaça. The PD from the wood have been identified and quantified (Almeida et al., 2010; Araruna et al., 2013). It is known that, together with several structures that occur in smaller proportions, coumarin represents over 50% of the phenolic derivatives extracted by ethanol. Therefore, coumarin is an important marker for cachaça stored in amburana barrels. In this work, a method is proposed for the routine monitoring of coumarin content, and it is indicated as a reference for characterizing the storage of cachaça in amburana barrels.

2. Materials and Methods

2.1 Preparation of cachaça aged with various types of wood

Samples of cachaça stored for two years in 500-L amburana barrels were analyzed as they were made available by the producers. Amburana chips and those of other types of wood ─ Ipê (Handroanthus albus), Garapa (Apuleia leiocarpa), Jatobá (Hymenaea courbaril), Jequitibá (Cariniana), Balsam (Myroxylon balsamum) and Oak (Quercus) ─ were supplied by a local cooper. The oak chips were obtained from the refurbishment of used European oak barrels.

2.2 Preparation of wood extracts

Wood extracts were prepared using two procedures: (a) direct extraction by the addition of 10.0 g of the respective chips to 200 mL of hydroalcoholic solution (50, 70 or 95%), followed by shaking on a shaking table for 12 h and filtering the extract through a fine screen; (b) extraction in a Soxlhet extractor, in which 10.0-g samples were added to extraction cartridges and maintained under reflux with 200 mL of the hydroalcoholic solvent (50, 70 or 95%) for 14 hours. The final volume of the extract was measured and, when necessary, completed to 200 mL. Wood chips were nearly always used directly to prepare the extracts without treatment. In some cases (as indicated), the chips were previously subjected to charring. For this purpose, they were humidified with 80% hydroalcoholic solution and heated over a flame for 3 to 4 minutes in a stainless steel container. The flame was extinguished, and the chips were allowed to dry by direct exposure to ambient air.

2.3 Analysis of cachaça and wood extracts

The quantitative analyses of the cachaças and extracts were achieved by adapting previous studies (Dias et al., 1997, Canuto & Silveira, 2006) using a Shimadzu model LC-

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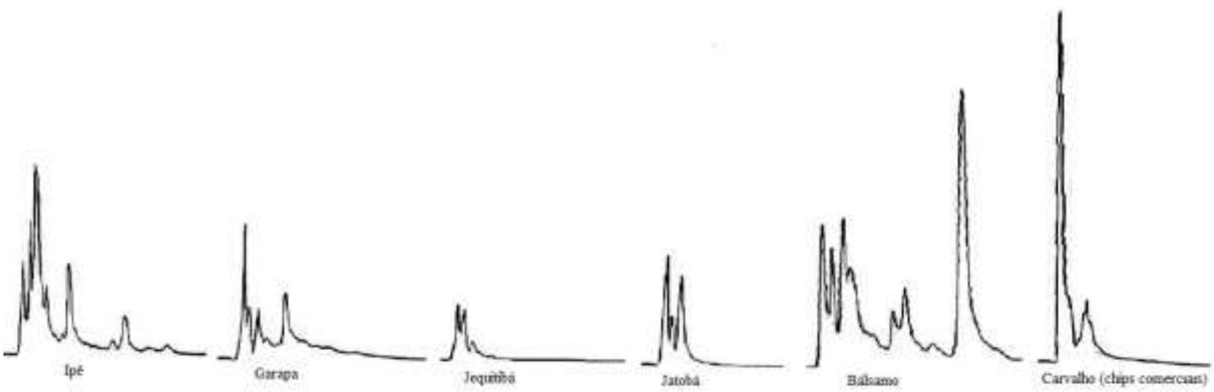
10AD high-performance liquid chromatograph with two pumps, a Shimadzu SPD-10AV UV-Vis detector, an RP18 column and a GODS (4) ODS 8345 pre-column. A manual injection system with a 100-µL loop, flow rate of 1.0 mL min^{-1} and an oven temperature at 40 ºC were employed. The mobile phases was composed of deionized water (60%) and acetonitrile (40%). The absorbance was measured at 274 nm. The total run time was 15 min. Coumarin was identified by the retention time and quantified using a standard curve. Other PC standards (gallic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, vanillic acid, anthraquinone, dihydrobenzofuran-2,3, dihydrocoumarin, scopoletin, guaiacol, kaempferol, pyrocatechol, quercetin and vanillin) were tested. Before injection into the chromatograph, the aliquots of the extracts were filtered through a 0.45-µm membrane and diluted 1:4 in the corresponding extraction solvent.

3. Results and Discussion

3.1 Analysis of wood extracts

The chromatograms of the extracts of the various types of wood in 50% ethanol are presented in Figure 1. The chromatographic profiles were very distinct and potentially applicable for the identification of each wood. Although peaks coincided in some retention time ranges (Table 1), the relative proportions between the peaks of each extract were well differentiated.

Figure 1. Chromatographic profile of the extracts of various types of wood in 50% ethanol.



Source: Authors.

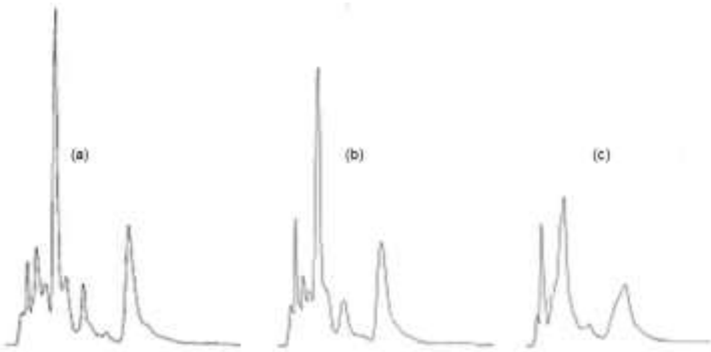
Table 1. Ranges of retention times of the peaks corresponding to the extacts of various types of wood in 50% ethanol.

Retention time (min)	Amburana	Bálsam	Oak	Garapa	Ipê Amarelo	Jatobá	Jequitibá
1.9 - 2.1	+	+	+	+	+	+	+
2.5 - 2.7	+	+	+	+	+	+	+
2.9					+		
3.4 - 3.5	+	+	+	+		+	+
3.7 - 3.8					+		
4.1 - 4.2	+	+		+			
4.8					+		
5.1 - 5.2	+						*trs.*
5.9 - 6.0	+			+			
6.7					+		
7.2		+					
7.4					+		
8.0 - 8.1	+	+					
10.1-10.2	+	+					
12.2	+						
12.5		+				+	
13.8					+		
					+		

Source: Authors.

The chromatograms of amburana extracts prepared with ethanol in three concentrations: 50, 70 and 95% (v/v) are presented in Figure 2. The results are representative of samples analyzed between 24 and 48 h after preparation and of replicates performed after 30 and 90 days (extracts kept in glass bottles). There is a good similarity between the profiles obtained with 50 and 70% ethanol in terms of the number of peaks and respective areas. In the 95% ethanol extract, the peaks were smaller and fewer. These observations are consistent with the colors of the extracts (Figure 3). Clearly, for the identification of a vat's wood on the basis of the chromatogram of the respective alcoholic extract (or of the cachaça stored in barrels made of that wood), one must have chromatographic profiles previously obtained for extracts of equivalent alcoholic content.

Figure 2. Chromatographic profiles of the extacts of amburana in ethanol (a) 50%, (b) 70%, (c) 95%.



Source: Authors

Figure 3. Extracts of amburana in 50, 70 and 95% (v/v) ethanol.

Source: Authors.

Among the woods analyzed, the characteristic and large peak of coumarin at 5.2 min. was only observed in the amburana extract (Table 1). This result corroborates the work of Santiago et al. (2017), who analyzed cachaças stored for 12 months in oak, amburana, jatobá, balsam and peroba barrels and detected coumarin (49.4 mg L^{-1}) in the cachaça stored in amburana barrels, and only traces of this compound in the cachaça stored in balsam barrels (1.7 mg L^{-1}); in the other barrels, the coumarin content was below the detection limit.

The extraction of PD by reflux did not change the chromatographic profile characteristic of amburana. The profiles maintained the same typical format at the three alcohol levels tested, as is shown in Figure 4 for the extraction with 70% ethanol. This result is consistent with Araruna et al. (2013), who reported equivalence between the chromatographic profiles of the alcoholic extract of amburana obtained before and after heating and even after drying to obtain the solid extract.

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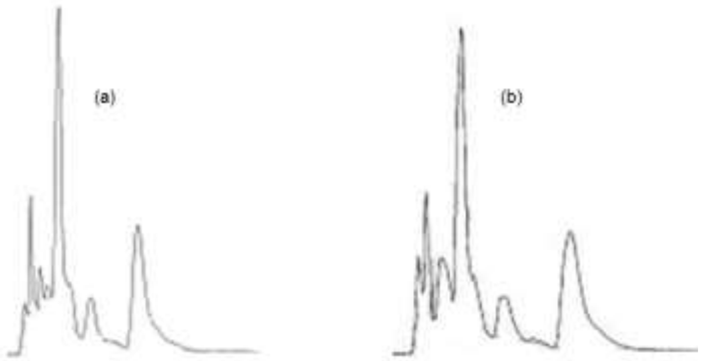
Figure 4. Chromatograms of extract of amburana chips with 70% ethanol (a) Direct room temperature extraction; (b) Reflux extraction with the solvent.



Source: Authors.

In all the extracts, the peaks corresponding to coumarin were sharp and narrow, with no indication of interference. In comparison with the available standards, the coumarin response coefficient was eleven times greater than that of scopoletin (6.2 min) and six times greater than that of dihydrocoumarin (3.8 min.).

A peculiar and prominent peak at 12.8 min was also observed in the chromatographic profiles. By comparison with the tested standards, this peak corresponded with that of vanillic acid, indicated by Santiago et al. (2017) as the PD with the second highest occurrence in cachaça stored in amburana barrels. Based on previous work, it can be assumed that this peak is related to 4-methylumbelliferone or to specific amburosides (Leal et al., 2006.;' Almeida et al., 2010.; Santiago et al., 2017).

Considering that charring is traditionally used to "revitalize" the inner part of the barrel staves, we investigated the possibility that this procedure would lead to changes in the characteristic profile of the amburana extract. Flakes of amburana previously used to obtain alcoholic extracts by maceration were charred and then subjected to a second extraction in 50, 70 and 95% ethanol. No relevant differences were found between the chromatographic profiles of the extracts of the raw chips and the flamed chips. However, based on peak areas, the reuse of charred chips resulted in reductions of the order of 30%, which varied according to each peak compared (data not shown). The decrease in the coumarin content is shown in Table 2.

Table 2. Coumarin content in amburana chip extracts (5% m/v).

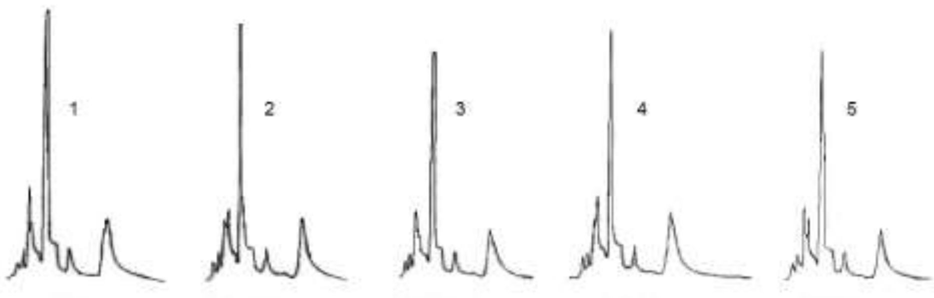
Alcohol content	Coumarin content (mg L^{-1})		Decrease in extraction (%)
	1st extract (raw chips)	2nd extract (flamed chips)	
50	300,6	203,4	32,4
70	332,2	217,3	34,6
95	303,8	185,5	38,9

Source: Authors.

3.2 Cachaça stored in amburana barrels

Chromatographic profiles similar to those of 50% and 70% ethanol extracts were observed in samples of cachaça stored by the same producer in five barrels of amburana for 24 months (Figure 5). On the average, the same peaks characteristic of hydroalcoholic extracts added up to 82% of the total area of the peaks, and the coumarin content was 31.5±1.9 mg L^{-1}.

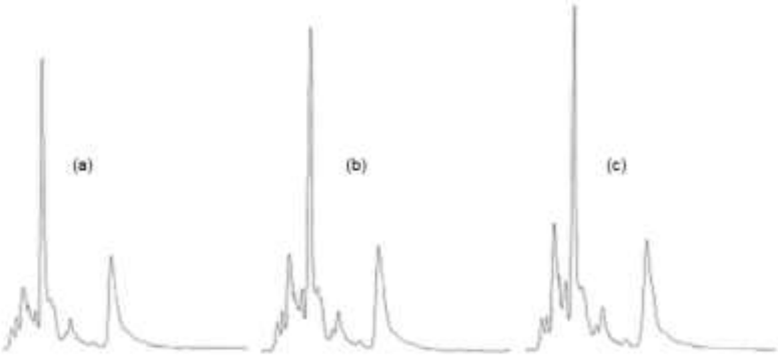
Figure 5. Chromatographic profiles of cachaças from five barrels of amburana (same producer).



Source: Authors.

The chromatographic profiles of samples from a second producer were also compared. These samples were collected after 24, 28 and 32 months from a new 500-liter (first use) amburana barrel (Figure 6).

Figure 6. Chromatographic profiles of a cachaça stored in 500-L amburana vats for 24, 28 and 32 months.



Source: Authors.

In the interval of eight months, there were noticeable increases in the areas of all the peaks, especially in the region prior to the coumarin peak. During this period, the average coumarin content was 189.6 ± 9.2 mg L^{-1}, much greater than the previous samples. In taste tests, samples were considered excessively woody and unfit for consumption. Coumarin is not carcinogenic and has a low toxicity compared to related compounds. However, warnings of risks associated with liver and kidney poisoning are associated with routine consumption (Leal et al., 2003; Leitão, 2009; Araruna et al., 2013; Grijalva et al., 2016). In fact, the European Community has established the limit of 10 mg L^{-1} for coumarin, although it admits higher limits for several other foods (CE, 2008). Thus, depending on the history of the vat and storage conditions, the standardization of "cachaça de amburana" might require important adjustments for sensory optimization and adaptation to safety criteria.

4. Conclusion

The definition of parameters to certify the wood used in cachaça storage tanks is an important step towards technological advances linked to the enhancement of native Brazilian flora. It was shown that a simple test that permits the identification of amburana (*Amburana cearensis*) as the wood used in the storage container and the monitoring of the evolution of its main phenolic compound (coumarin) is an important reference in the standardization procedures of the beverage. The aging of the beverage in wooden barrels is the long phase of the cachaça production process, involving periods ranging from twelve months to several years. Innumerable chemical components are added to the beverage at this stage, which are not yet declared, nor are they subject to laboratory monitoring, either by the producers or by the inspection organ. It is hoped that this work will stimulate further studies so that the standardization of analytical methodologies for the identification of the wood that comprise the barrels is feasible, and that the establishment of limits related to the contents of certain bioactive components incorporated into the beverage will occur. The fact that *Amburana cearensis,* like other species, is an endangered species must be emphasized (Iucn, 2020). To expand its use in the aging of cachaça, as well as that of other Brazilian species, it is essential to validate, within the scope of the legislation, some alternative procedures that have been proposed based on the exclusive use of the bark, branches or chips from the plant.

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Lorena Simão Marinho – 35%

David Lee Nelson – 20%