Evolution of the content of phenolic compounds, antioxidant activity and color in organic sugarcane spirit aged in barrels of different woods

Evolução do teor de compostos fenólicos, atividade antioxidante e cor em cachaça orgânica envelhecida em barris de diferentes madeiras

Evolución del contenido de compuestos fenólicos, actividad antioxidante y color en cachaça orgánica envejecida en barreras de diferentes maderas

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Abstract

During the aging of sugarcane spirit several chemical reactions occur. Therefore the aim of this work was to study the evolution of phenolic content, antioxidant activity and changes in color in organic sugarcane spirit aged in different woods for twelve months. The sugarcane spirit aged in barrels of jatobá showed higher levels of phenolic compounds and antioxidant activity (277.3 mg EAG.100 g^{-1} and -7.749 $mg.L^{-1}$), followed by sassafras (83.8 mg EAG.100 g^{-1} and -0.299 $mg.L^{-1}$) and ipê (67.6 mg EAG.100 g^{-1} and 4.180 $mg.L^{-1}$). The colorimetric variables (brightness, chroma and metric hue angle) were significantly influenced by the species of the woods.

Keywords: Barrels; Sassafras; Jatobá.

Resumo

Durante o envelhecimento da cachaça, ocorrem várias reações químicas. Portanto, o objetivo deste trabalho foi estudar a evolução do conteúdo fenólico, atividade antioxidante e alterações na cor da cachaça orgânica envelhecida em diferentes madeiras por doze meses. A cachaça envelhecida em barris de jatobá apresentou maiores níveis de compostos fenólicos e atividade antioxidante (277,3 mg EAG.100 g^{-1} e -7.749 $mg.L^{-1}$), seguida de sassafrás (83,8 mg EAG.100 g^{-1} e - 0,299 $mg.L^{-1}$) e ipê (67,6 mg EAG.100 g^{-1} e 4.180 $mg.L^{-1}$). As variáveis colorimétricas (brilho, croma e ângulo métrico da tonalidade) foram influenciadas significativamente pelas espécies da floresta.

Palavras-chaves: Barris; Sassafrás; Jatobá.

Resumen

Durante el envejecimiento de la cachaça, se producen varias reacciones químicas. Por lo tanto, el objetivo de este trabajo fue estudiar la evolución del contenido fenólico, la actividad antioxidante y los cambios en el color de la cachaça orgánica envejecida en diferentes maderas durante doce meses. La cachaça envejecida en barriles de jatoba mostró niveles más altos de compuestos fenólicos y actividad antioxidante (277.3 mg EAG.100 g-1 y -7.749 mg.L-1), seguido de sasafrás (83.8 mg EAG.100 g- 1 e - 0.299 mg.L-1) e ipe (67.6 mg EAG.100 g-1 y 4,180 mg.L-1). Las variables colorimétricas (brillo, croma y ángulo de sombra métrico) fueron influenciadas significativamente por las especies forestales.

Palabras clave: Barriles; Sasafrás; Jatobá.

1. Introduction

The aging in wood barrels is one of the most important stages of getting aged sugarcane spirit of *premium* and *extra premium* types (Brasil, 2005). The reactions that occur

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during aging favor the formation of compounds that affect the color, odor and taste of liquor (Mendes et al., 2002).

Studies with high quality distillates point aging as essential for such drinks reach this condition due to numerous changes in the profile of congeners occurred over this period. The congeners that provide improvements in sensory aspects includes acids, aldehydes, tannins and other compounds, and are normally referred to as phenolic compounds of low molecular weight (Mangas et al., 1996). Some of these compounds are adopted as "Markers" or "Aging Patterns" because by monitoring the qualitative and quantitative changes of them is possible to estimate the distillate aging time (Lo Coco et al., 1995; Mangas et al., 1996; Jaganathan & Dugar, 1999), since the formation of most of these compounds is based on the oxidative degradation of lignin by ethanolysis in the presence of molecular oxygen during storage of the beverage into barrels. Generally, vanillin and coniferaldehyde are obtained in larger amount of lignin derived from softwoods, while syringaldehyde and sinapaldehyde result of lignins hardwoods (Piggott et al., 2003).

Recently, Goldberg et al. (1999) addressed an important aspect of the phenolic constituents of low molecular weight present in aged drinks, highlighting the antioxidant activities, scavenging free radicals, anticarcinogenic, anti-inflammatory. Antioxidants are compounds that can retard or inhibit the oxidation of various substrates, from single polymer molecules to complex polymers and biosystems, preventing the onset or propagation of chain reactions in the oxidation process (Angelo & Jorge, 2007).

The antioxidant activity of the phenolic compounds depends on their chemical structure, concentration and oxidation state, which are mainly determined by the aging condition, including the barrel characteristics, such as the botanical species, pretreatment of the barrel, size and environmental storage conditions (Canas et al., 2008; Alañón et al., 2011a).

Several studies have been conducted in order to test different woods for sugarcane spirit aging, characterizing and quantifying the phenolics present and their antioxidant activities, searching woods that have similarity with the traditionally used as an oak (Alañón et al., 2011b).

Given the rich brazilian flora and considering the above, this study aimed to identify the main changes in the levels of phenolic compounds, antioxidant activity and changes in color in organic sugarcane spirit aged in barrels of yellow Ipê (*Tabebuia sp.*), jatobá (*Hymenaea sp.*) and sassafrás (*Ocotea sp.*) for twelve months of storage.

2. Materials and Methods

The present work is a quantitative study, carried out in a laboratory (which included the aging of sugarcane spirit, the determination of phenolic compounds, antioxidant activity and colorimetric variables and data analysis) (Pereira et al., 2018).

The raw material used was aged sugarcane spirit not produced by organic system, single distilled in copper stills from the company Alambique Cambéba headquartered in the city of Alexânia – GO, sugarcane spirit used in this experiment represents the heart fraction of the distillation process. The treatments were based on the aging of sugarcane spirit in barrels of ipê, jatobá and sassafrás for 12 months. Was adopted a completely randomized design with three treatments and four replications made of barrels of the same botanical species.

The barrels contained volume of 20 liters. Details on the size of the barrels and gallons are in Annex A (Viana, 2007). The physicochemical characteristics of the wood used in the experiment are in Annex B (Lorenzi, 2002; Mori et al., 2006).

The experiment was carried out on the premises of the Agronomy School of the Federal University of Goiás, for 12 months, between the months of December 2016 and January 2018. The sugarcane spirit without aging had 41% alcohol, aging was conducted in a room under controlled temperature (21 ± 5ºC) and relative humidity of 65 ± 10 ºC, protected from sunlight and vibrations. Monthly samples were collected and packed in amber glass vials and sent for analysis. Analysis of phenolic compounds, antioxidant activity and color were made.

2.1 Total phenolic compounds

The quantification of the total phenolic compounds of the samples was performed according to the *FolinCiocalteau* method, this method is based on reducing phosphomolybdic and phosphotungstic acids by the phenolic compounds of the samples with the development of a blue color in alkaline solution, whose intensity increases 760 nm (Singleton, 1995).

To perform the analysis was added 2.5 mL of *FolinCiocalteau* 10% solution and 2 mL of a sodium carbonate 7.5% solution and 0.5 mL of each sample in test tubes. The mixture was allowed to stand for 2h. The reading of the samples was made in a spectrophotometer Ultrospec 2000 brand, model SP-220, white being a mixture of ethyl alcohol, *FolinCiocalteau* reagent and Sodium Carbonate used in the same proportions to the samples. The amount of total phenols of each sugarcane spirit sample was quantified by performing a standard curve prepared with gallic acid. For the preparation followed the same procedure but replacing the sample by different concentrations of gallic acid, ranging from 50 $mg.L^{-1}$ to 400 $mg.L^{-1}$. Thus, the total phenolic compounds of the samples were expressed as gallic acid equivalents (GAE) and analysis performed in triplicate.

2.2 Antioxidant activity

The antioxidant activity of sugarcane spirit was determined by the blocking effect of free radicals (DPPH) (Alañón et al., 2011a; 2011b). The method evaluates the reducing power by the methodology of DPPH (2,2-diphenyl-1-picrylhydrazyl), extracts were first prepared with different concentrations of sugarcane spirit, these were obtained by measuring the volume of 0.5 mL, 1 mL, 2 mL, 3 mL, 3.5 mL and 4 mL of liquor and completed the final volume with distilled water to 4 mL. After obtaining the extracts were removed and 0.4 mL added to these test tubes and with 3.6 mL of 0.06 mM DDPH reagent. The test tubes were shaken vigorously, then the solution was rested for 60 minutes in the dark. At this time the absorbance was measured at 517 nm of the solutions. All analyzes were performed in triplicate.

The blocking effect of DPPH was calculated from the percentage of DPPH discoloration by using the following equation 1:

$$\text{Effect blocker } (\%) = \left[\frac{A_{DPPH} - A_S}{A_{DPPH}}\right] \text{ x } 100 \qquad (1)$$

At where: A_S = Absorbance of the sugarcane spirit of the sample; A_{DPPH} = Absorbance of the DPPH solution.

For the IC_{50} calculation was used the method described by Santos et al. (2011) with adaptations, the 50% effective concentration express the minimum antioxidant concentration required to reduce by 50% the initial concentration of DPPH. From the absorbances obtained from the different dilutions of the extracts (0.5 mL, 1 mL, 2 mL, 3 mL, 3.5 mL and 4 mL), plotted is the % reduction of DPPH on the “y” axis, and it was determined the equation 2 of the line.

$$y=-ax+b \qquad (2)$$

At where: y= % reduction of DPPH; x= IC_{50} (mg/mL).

To calculate the IC_{50} was used the straight line equation by substituting the value of “y” by 50 to obtain the sample concentration capable of reducing the 50% DPPH. The extracts with lower IC_{50} have higher antioxidant activity.

2.3 Color

To check changes in color during the 12 months of research, analyzed in triplicate sugarcane spirits through the parameters L*a*b* or CIELab, where L* represents the brightness, expressed as a percentage (from 0 to black 100 for white), a* and b* are two color ranges going respectively from green to red and from blue to yellow with values ranging from -120 to +120. From the values of L (brightness), a (- green / + red) and (- blue / + yellow) values were determined: metric chroma (c) and hue angle (h). Analyses carried out in order ColorQuest II color spectrophotometer / Hunter Lab. The apparatus was set in reflectance with specular included, using standard white nº C6299 of 03/96 and clean glass sample cuvette of 10 mm pathlength, 1-inch field of analysis.

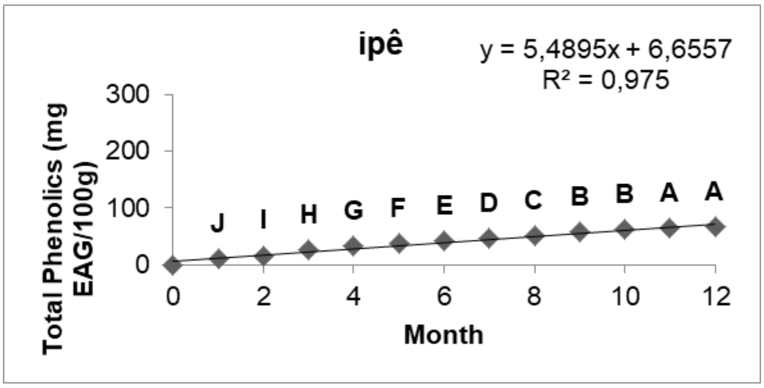
The results obtained in the experiment were assessed by analysis of variance (ANOVA) and the mean test used the Scott-Knott test for it was used EXCEL and R software (R Development Core Team, 2013). The graphs were plotted from the average obtained in repetitions for each parameter evaluated.

3 Results And Discussion

3.1 Total phenolic compounds

Aged sugarcane spirit in jatobá wood had higher average levels of total phenolic compounds, followed by sassafras and ipê (Figure 1).

Figure 1. Evolution of the average content of total phenolic compounds of barrels of organic sugarcane spirit without aging and after aging in barrels of ipê, jatobá and sassafrás over 12 months, with their differences by Scott-Knott test ($p<0.05$).



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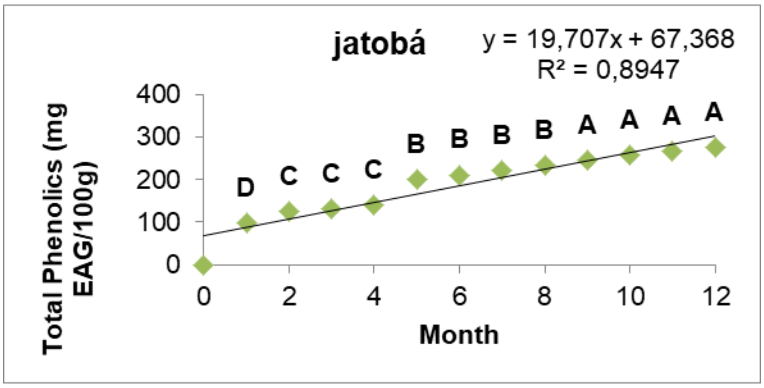

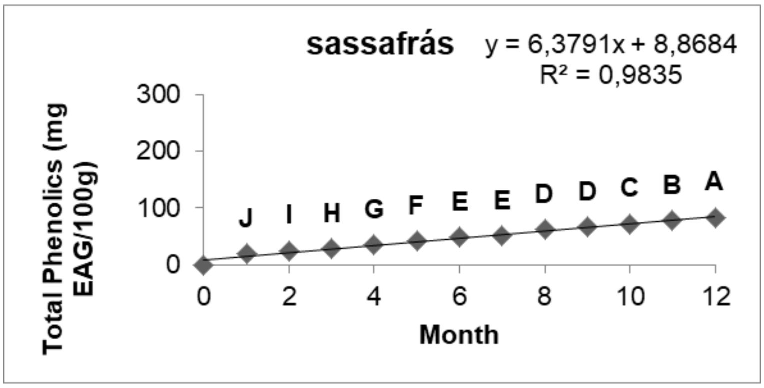

Source: Author (2020).

The variation in results is a reflection of the intrinsic characteristics of each wood, for each wood species include amount and different types of phenolic compounds, most commonly found are: ellagic acid, sinapaldehyde, syringaldehyde, coniferaldehyde, vanillin, vanillic acid, syringic acid, catechin, epicatechin, coumarin, mirecetina, trans-resveratrol and eugenol. However the amount and presence of these depend on the botanical species used in the manufacture of the barrels (Silva et al., 2012).

Thus in the present study we observed increase in the content of phenolic compounds as a function of aging time, as the organic sugarcane spirit showed no lack of phenolic compounds in its composition, while in the end of the aging period organic sugarcane spirit showed increases of 67.6 % in barrels of ipê, 277.3 % in barrels of jatobá and 83.8 % in barrels of sassafrás in the content of phenolic compounds.

Changes in phenolic composition of sugarcane spirit during the aging period has been studied by several authors as Parazzi et al. (2008) who studied the chemical compounds of sugarcane spirit aged in oak barrels (*Quercus sp.*), the authors observed that occurred near 45% increase in the levels of phenolic compounds as a function of time in spirits stored in wooden barrels, but the spirits stored in glass containers there was no incorporation of these compounds during the aging period. This behavior was also observed by Dias et al. (1998),

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Aquino et al (2006) and Cardoso et al. (2008) the increased content of phenolic compounds occurs due to the progressive incorporation of components from the wood to the beverage, making it yellow and softer taste, attenuating the dehydrating alcohol presence feeling (Mendes et al., 2002). The aging results of organic sugarcane spirit stored in the barrels of ipê, jatobá and sassafrás during 12 months follow this tendency, therefore agrees with the results found by the authors, suggesting that also occurred incorporation of wood compounds in all treatments, resulting in increased phenolic compounds content of the beverage.

3.2 Antioxidant activity

The extracts with lower IC_{50} values have a higher antioxidant potential, therefore analyzing the data relating to antioxidant potential show in Table 1, it can be seen that the highest antioxidant activity was observed in aged sugarcane spirit in jatobá (-7.749 $mg.L^{-1}$) followed by sassafrás (-0.299 $mg.L^{-1}$) and last ipê (4.180 $mg.L^{-1}$).

Table 1. Equations used to calculate IC_{50} antioxidant activity and average results of IC_{50} antioxidant capacity of organic sugarcane spirit aged in barrels of three different woods, ipê, jatobá and sassafrás for 12 months.

Wood	Month	Line Equation	R^2	* IC_{50} (mg/L)
Ipê	1	y = 5,0221x + 4,4332	0,9329	9,073
	2	y = 5,9048x + 4,9968	0,952	7,621
	3	y = 6,4637x + 5,5475	0,9439	6,877
	4	y = 6,7466x + 4,5721	0,9806	6,733
	5	y = 6,4892x + 5,8268	0,9783	6,807
	6	y = 6,4479x + 7,4946	0,9635	6,592
	7	y = 6,492x + 8,3644	0,9575	6,413
	8	y = 6,2833x + 10,425	0,9453	6,298
	9	y = 6,0993x + 12,996	0,9481	6,067
	10	y = 5,7435x + 16,522	0,9668	5,829
	11	y = 5,4537x + 18,324	0,9509	5,808
	12	y = 5,1335x + 28,54	0,9764	4,180
Jatobá	1	y = 2,6278x + 28,911	0,9428	8,025
	2	y = 3,7602x + 38,986	0,9291	2,929
	3	y = 3,7436x + 40,51	0,9401	2,535
	4	y = 3,4654x + 45,602	0,9756	1,269
	5	y = 2,6279x + 52,467	0,9806	-0,939
	6	y = 2,6448x + 56,282	0,9953	-2,375
	7	y = 3,6238x + 56,918	0,946	-1,909
	8	y = 3,3054x + 63,308	0,989	-4,026
	9	y = 3,8055x + 62,573	0,9852	-3,304
	10	y = 2,9981x + 69,816	0,9506	-6,610
	11	y = 3,0735x + 71,3	0,926	-6,930
	12	y = 3,0572x + 73,689	0,9541	-7,749
sassafrás	1	y = 3,9051x + 18,636	0,9702	8,032
	2	y = 3,8932x + 20,554	0,9848	7,563
	3	y = 3,5087x + 24,54	0,9664	7,256
	4	y = 4,0883x + 21,68	0,9333	6,927
	5	y = 4,2655x + 22,998	0,9658	6,330
	6	y = 4,1223x + 27,457	0,9545	5,469
	7	y = 3,6025x + 32,29	0,9429	4,916
	8	y = 3,2575x + 37,044	0,9247	3,977
	9	y = 4,4188x + 34,688	0,9226	3,465
	10	y = 3,816x + 40,666	0,9545	2,446
	11	y = 3,8895x + 43,353	0,961	1,709
	12	y = 3,0863x + 50,922	0,9329	-0,299

* IC_{50}: minimum concentration of antioxidant required to reduce by 50% the initial concentration of DPPH. Source: Author (2020).

It was also observed that the slope was positive for all treatments, the slope was growing, that is, the antioxidant potential increased as the reactions have developed over the twelve months of age, R^2 found in treatments ranging for barrels of ipê from 0.9329% to 0.9806%, for barrels of jatobá 0.9428% to 0.9953% and for the barrels of sassafrás 0.9702%

to 0.9848%, as the calculated values for R^2 are near to one, it means that the degree of adjustment of the straight section can be considered good because as calculated for this study variable (x) accounts for about 95% variable (y) (Tryola, 2008).

Studies show that differences in antioxidant potential may be higher if the analyzed sample is food, since it is a complex matrix of different components, which can agree among themselves many different interactions (Pérez-Jiménez & Saura-Calisto, 2006). According to Miranda et al. (2007), the antioxidant activity is also influenced by factors such as: wood species, size and pre-treatment of the barrel, environmental conditions, aging time and alcohol content of the beverage. Alañón et al. (2011b) confirm the observations of Miranda et al. (2007), they perceived in their study, the botanical species used for the manufacture of barrels, influence the content and quality of phenolic extracts as well as its antioxidant activities. These results agree with those obtained by Aoshima et al. (2004), who evaluated the antioxidant activity in various types of whiskeys by DPPH method. The authors (Aoshima et al., 2004) observed that different types of whiskey stored under different conditions, such as type of barrel, sort of oak, or pre-treatment of the wood, showed differences in antioxidant activity for each wood.

Given that all treatments of this work started from the same sample (organic sugarcane spirit without aging), the results of this study are consistent with the points made by the authors, suggesting that the values found for antioxidant activity ranged from one wood to another because the intrinsic characteristics of each wood.

Other factors contributing to the results found are the presence of phenolic compounds present in aged sugarcane spirit (Figure 1). These compounds have antioxidant activity and we can see that the samples which had higher concentrations of total phenolic compounds also showed higher antioxidant activities.

3.3 Color

The results show that organic sugarcane spirit stored in ipê, jatobá and sassafrás barrels showed significant positive change in brightness (L) over the 12 months of aging (Table 2), that is, sugarcane spirit was clearing over that period.

Table 2. Averages of colorimetric variables of aged organic sugarcane spirit in barrels of ipê, jatobá and sassafrás over 12 months, with their differences by Scott-Knott test (p<0.05).

Wood	Month	L	C	h
Control	0	31,1	0,09	5,71
	1	65,6e	6,49d	0,24b
	2	66,5d	7,12d	0,33b
	3	66,8d	7,62c	0,44b
	4	74,2c	5,16e	-0,53c
	5	74,1c	9,66b	-0,22c
ipê	6	73,3c	11,91a	-0,13c
	7	73,8c	3,36f	-0,59c
	8	75,4b	6,40d	-0,60c
	9	75,3b	8,06c	-0,48c
	10	75,8b	9,45b	-0,39c
	11	74,4c	11,55a	-0,31c
	12	81,9ª	6,64d	1,06a
	1	67,20e	67,20e	0,50b
	2	68,49d	68,49d	0,63b
	3	68,91d	68,91d	0,76b
	4	74,90c	74,90c	-0,37d
	5	74,46c	74,46c	-0,18c
jatobá	6	73,94c	73,94c	-0,12c
	7	76,43b	76,43b	-0,71e
	8	77,82b	77,82b	-0,45d
	9	77,62b	77,62b	-0,34d
	10	76,93b	76,93b	-0,28d
	11	74,91c	74,91c	-0,37d
	12	83,98ª	83,98a	1,49a
	1	64,56e	3,27c	0,28c
	2	64,93e	3,73c	0,22c
	3	64,75e	4,35c	0,17c
	4	71,26d	2,62d	-0,68f
	5	73,18c	7,70b	-0,16d
sassafrás	6	72,64c	10,65a	-0,05d
	7	70,52d	1,16d	1,33a
	8	74,95b	4,71c	-0,55f
	9	74,19b	6,89b	-0,30e
	10	74,69b	7,91b	-0,27e
	11	73,55c	8,26b	-0,29e
	12	80,70ª	2,98d	0,55b

L: luminosity, C: metric chroma and h: tone. Source: Author (2020).

The hue angle (h) in which distinguishes the coordinate a* (green - / red +) of b* (blue - / yellow +), showed significant variations during the aging period, were observed values indicating an inclination added to the positive a* coordinate with respect to negative b* coordinate, that is, closer to yellow to red pigmentation of values, showing the sugarcane spirit takes on a more reddish color during the months of aging. The metric chroma (c) varied significantly during storage for al evaluated woods, resulting in increased intensity of chroma of aged sugarcane spirit in ipê, jatobá and sassafrás.

Each wood provides taste, odor and color. These factors can also vary within the same wood, depending on the time of use and the barrel size (Trindade, 2006). This was observed in this experiment since the color coordinates are changed every month during the entire time.

The study reveals that regardless of wood with the barrel was built, aged organic sugarcane spirit presented darker compared to the original (organic sugarcane spirit without aging). According to Mendes et al., (2002), sugarcane spirit under aging in wood becomes yellowish due to the progressive extraction of phenolic compounds from wood barrel. Furthermore, according to Singleton[16], phenolic compounds may react with copper from the distillate, promoting blackening of the beverage. This information consistent with the results found in this study, because the aged sugarcane spirit show incorporation of phenolic compounds (Figure 1) during the storage period and hence a darker coloring end.

4. Conclusion

At the end of the period of aging was concluded that:

The aging process caused in organic sugarcane spirit stored in different woods the incorporation of phenolic compounds and increase in the antioxidant potential of the beverage, and the barrels made of jatobá wood showed higher levels of phenolic compounds and antioxidant activity (277.3 mg EAG.100 g^{-1} and -7.749 $mg.L^{-1}$), followed by the sassafrás (83.8 mg EAG.100 g^{-1} and -0.299 $mg.L^{-1}$) and ipê (67.6 mg EAG.100 g^{-1} and 4.180 $mg.L^{-1}$). Regarding the color of the organic sugarcane spirit all woods provided during the aging process the darkening of the beverage. It is suggested that in future works use other woods, with the aim of adding value and quality to cachaça.

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