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Full Length Research Paper

Evaluation of maturation congeners in beer aged with Brazilian woods

Bernardo Pontes Guimarães, Luis Eduardo Pereira Neves, Munique Gonçalves Guimarães and Grace Ferreira Ghesti*

Laboratory of Brewing Bioprocesses and Catalysis Applied to Renewable Energies, Institute of Chemistry, University of Brasília, caixa postal 4478, Brasília-DF, 70904-970, Brazil.

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Beer is the most consumed alcoholic beverage in Brazil. In this paper, wood aged beer was made with some Brazilian wood species, that is, chestnut, *Cariniana* sp., balsam, oak, and amburana. This maturation process can be done with wooden cubes, chips or in barrels. The main differences between the processes are related to the surface area of wood in contact with the beer as well as where it is stored. They provide different sensorial and visual characteristics, such as turbidity, colour, and their effects on drinkability. In view of the great variety of wood in Brazil, one may wonder about their application to this end; however, for the purpose of maturation it is crucial to collect data regarding the characterisation of several types of wood. Brazilian wood samples were analysed in order to use their cubes in a lager beer sample. Thus the objective of this study was the chemical characterisation of roasted wood so they could be applied as a technological innovation in the maturation process to produce wood aged beers. In order to do so, the wood's physicochemical and sensorial effects on the beer were compared to their effects on a standard base beer. It was observed that they have characteristic flavours and aromas that come from the wood's structural compounds, through the thermal degradation of lignin and holocellulose. The trained panel related increasing the complexity of the beer lagered with wood. Physicochemical and High Performance Liquid Chromatography (HPLC) analyses verified the samples' sensorial results. According to the results, lagering with wooden cubes is an effective technique to be used in beer aging, influencing organoleptic characteristics.

Key words: Beer, wood aged drinks, wood aged beer, brewing technology, wooden cubes.

INTRODUCTION

Beer production in Brazil has been on the rise for the last 30 years. Recently it reached the 1.4 billion litres mark, making the country the third largest producer in the world. Not only the volume is on the rise, but also the number of craft breweries, from 356 in 2014 to 835 in 2018 (Dias

and Falconi, 2018) and with it different beer styles, such as wood-aged beers.

Historically, wines and spirits have used wood aging process longer than beer and so have the studies in this area. Maturation of distilled spirits is the main factor in

*Corresponding author. E-mail: ghesti.grace@gmail.com. Tel: +55 6192772610.

their characterisation. Approximately 60% of the aromatic compounds present in the beverages are either from the wood or its interaction with the distilled spirit, with the remaining percentage coming from the raw material or fermentation (Conner et al., 2003). Unaged spirit has strong sensorial characteristics and an intense alcoholic flavour, both of which can be attenuated by aging. There are countless physicochemical interactions between the wood and the spirit, in which many transport phenomena occur with both volatile and non-volatile compounds. The evolution of phenolic compounds, oxidation of the spirit, colour and flavour stabilisation, and the development of the wooden quality contribute towards the variety and complexity of the aroma components and, consequently, result in higher added values for distilled spirits (Conner et al., 2003; Bortoletto and Alcarde, 2015; Zhang et al., 2015).

In the brewing process, lagering makes the production more suitable to the formation of proteins-polyphenols complexes, which precipitate under low temperatures. This step of the process is characterised by secondary fermentation, beer saturation with CO₂, and extraction of all non-soluble compounds found in beer. The amount of time it takes can vary widely, from 7 to 30 days (conventional processes), or depending on the style of beer, it can take more than two years (Bortoletto and Alcarde, 2013).

According to some beer style guides such as the Beer Judges Certification Program (Strong and England, 2015) and Brewers Association (2019), the process of aging beer in wood is a traditional method, seldom used by large breweries and more commonly used in small breweries for the production of craft beers. The use and study of oak barrels are widely common, while studies of other types of wood to this end have become popular.

The greatest contribution of wood aging is related to compound extraction during aging and the interaction of these components to the ones originally found in the beverage. The aromatic profile resulting from the extractables depends on several factors, the main ones being the wood gender and species. Moreover, during this process many chemical reactions may occur, especially due to the direct extraction of the wood components, degradation of the cell wall's chemical compounds, and reactions between the wood compounds and the beverage (Anjos et al., 2011; Zhang et al., 2015).

Other factors are related to the different wood treatments made by cooperages, such as cut, maturation, and thermal treatment. The aging time and conditioning of the storage strongly impact the chemical quality and organoleptic properties of the final product (Conner et al., 2003). The lagering may be done in barrels that were previously used to age wine, whiskey or other spirits. In these scenarios, the beer lagered may acquire aromas and flavours of the original beverage storage in the barrel, as shown in Figure 1.

Countless chemical reactions are associated with the

aging process of sugar cane spirit (*cachaça*), the main being: reactions amongst the non-alcoholic volatiles from the distillation; direct extraction of compounds from wood; decomposition of wood macromolecules (lignin, cellulose, hemicellulose, etc.) and its incorporation by the spirit; transformation of the wood's extracted materials; reactions of the original spirit components; vaporisation of the volatile compounds through the wood of the container; formation of stable molecule complexes from secondary compounds and water and/or ethanol; amongst others (Anjos et al., 2011).

During spirit storage in wooden barrels, there is a progressive increase in dry extract, in which tannins and phenolic compounds from lignin may represent up to 40%. Lignin during storage suffers acid alcoholysis at room temperature, giving up vanillin, syringaldehyde, coniferaldehyde and guaiacol, eugenol and sinapaldehyde (Wylar et al., 2015; Silva et al., 2009). The degradation of hemicellulose and cellulose, present throughout all plant biomass, produces furfural and 5-hydroxymethylfurfural (5-HMF). In addition to these molecules, others have been found in beverages that have been aged in oak barrels, such as gallic acid, vanillic acid, syringic acid, coumarin and ellagic acid (Zacaroni et al., 2011; Santiago et al., 2014; Bortoletto et al., 2016).

It is well known that Brazil has a vast diversity of plants and is home to species of wood trees with heterogeneous structures and properties (Giulietti et al., 2005). Therefore, the country presents great potential for the use of native species for storing beverages; not only beer but also other alcoholic beverages that use the storage procedure of aging. Hence, studies and characterisation of the chemical composition of these alternative woods are fundamental to evaluate the insertion of such wood types in beer production, in addition to opening new possibilities of use in the cooperage and food industries, with emphasis on the alcoholic beverages industry.

In light of all the aforementioned facts, the goal of this work is to provide a physicochemical characterisation of beer aged with the following Brazilian wood types: amburana (*Amburana cearensis*), balsam (*Myroxylon balsamum*), Cariniana (*Cariniana* sp.) and chestnut tree (*Bertholletia excelsa*), comparing them to the most frequently wood used for this purpose, oak (*Quercus* sp.). The modifications that occurred to the beer samples were then evaluated through physicochemical, microbiological, and sensorial analyses.

MATERIALS AND METHODS

Beer (neutral lager beer, alcohol content 6,60%, pH 4.4) was produced in a single batch and kindly provided by Cervejaria Embuarama (Brasília, Brazil). They were then stored in 30L-tanks with no wood (BL) or with 90 g of wood cubes (1 cm³) for 90 days at 0°C. The wood used in this study were Amburana (AM), Balsam (BA), Cariniana (CA), Chestnut (CN) and Oak (OA) (Dornas Havana, Brazil). For the wood cubes, the extractable content analysis was conducted in accordance with the TAPPI204 om-88

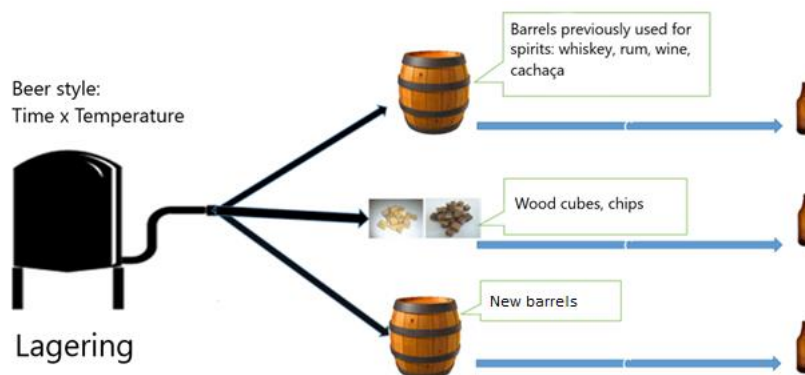


Figure 1. Types of wood utilisation in beer.

Table 1. Characterisation of amburana (AM), balsam (BA), Cariniana (CA), chestnut (CN) and oak (OA) regarding their main extractables.

Sample	Extractables (%)	Ashes (%)	Lignin (%)	Holocellulose (%)	Moisture (%)
AM	7.05	0.15	43.78	56.07	5.12
BA	16.57	0.75	44.32	54.93	5.20
CA	7.71	0.62	43.12	56.26	5.24
CN	7.6	0.41	37.05	62.54	4.37
OA	16.55	0.14	40.39	59.53	5.50

standard method. For the lignin content, the steps were performed according to the TAPP 204 om-88 standard (solvent extractives of wood and pulp). The ashes and moisture content were made in accordance to Miranda et al. (2020).

The acidity and relative density of the beer samples matured in wood were measured in accordance with the Association of Official Analytical Chemists – AOAC – for beverages and vinegar. The pH measurement of the beer matured in wood was carried out according to European Brewery Convention (EBC) Method 9.42. The colour analysis was measured at 430 nm, as indicated in EBC 9.6. The turbidity was analysed in a turbidimeter (ALFAKIT, Brazil) and the values as stipulated in nephelometric turbidity units (NTU). The alcoholic content was measured with PBA-B Generation M Modular Measuring System with AlcolyzerPlus Beer and density meter (DMA 5000 M) (Anton Paar, Austria). In order to determine the phenolic content, the UV-Vis spectrophotometry method by Folin-Ciocalteu was used. The absorbance was measured at 765 nm and the results expressed in mg/L, the standard used was gallic acid.

The maturation congeners were analysed using a high performance liquid chromatography system (Shimadzu, model LC-10AD, Kyoto, Japan) equipped with a UV-Vis detector as described by Wyler et al. (2015). The chromatographic standards were gallic acid, furfural, vanillin, vanillic acid, syringaldehyde, sinapaldehyde, syringic acid and coniferaldehyde with >99% purity. The High Performance Liquid Chromatography (HPLC) method employed two mobile phases composed of water-acetic acid (98:2, v/v) and methanol-water-acetic acid (70:28:2, v/v/v). The analytical parameters of the chromatographic analyses were determined according to a simple linear relationship.

The sensorial analyses were conducted at the University of Brasília (UnB), with a trained panel of analysts that was evaluated through theoretical and practical classes using the off-flavours kit

(FlavorActiv, United Kingdom) and trained by specific personnel authorised to do so. They were responsible for the evaluation of the samples presented in this paper. The sensorial analyses' results were divided in two groups for each sample: aroma and flavour. The analysis file had a pre-determined list of aromas and flavours characteristic of beverages matured in wood and a subjective option for flavours or aromas not listed but detected by the analyst. The file had a 1-5 grading system, where 1 was the least and 5 the highest sensorial perception. The arithmetic average of the panel's grades for each characteristic was calculated in order to analyse the data and the result expressed as a spider graph for visual representation.

RESULTS AND DISCUSSION

Wood characterisation

It is expected that woods with a higher extractable content will have a higher impact on the taste and physicochemical and organoleptic characteristics of beer. Wyler et al. (2015) in their paper on oak wood in beer reported the influence of wood is related to the storage time and the intensity of the chemical reactions that occur between it and the beverage. The most important contents were moisture, lignin, holocellulose and ashes, all having their share in influence and adding even more values and special flavours to beers.

As shown in Table 1, BA (16.57%) and OA (16.55%) samples showed the highest quantity of extractables,

Table 2. Relative density, alcoholic content and apparent extract of beer.

Sample	Relative density at 20°C	Alcoholic content (%, v/v)	Apparent extract (%, m/m)
BL	1.019 ± 0.001	6.60	3.94
AM	1.019 ± 0.002	6.59	4.07
CA	1.018 ± 0.002	6.64	3.94
CN	1.019 ± 0.012	6.64	3.96
BA	1.020 ± 0.000	6.58	4.05
OA	1.018 ± 0.001	6.61	4.01

Table 3. pH and total acidity in the beer.

Sample	pH	Total acidity (mg/L)
BL	4.45	0.704 ± 0.016
AM	4.48	0.730 ± 0.016
BA	4.45	0.672 ± 0.028
CA	4.50	0.681 ± 0.032
CN	4.51	0.668 ± 0.025
OA	4.46	0.802 ± 0.016

Table 4. Microbiological analysis of the beers (cfu/mL).

Sample	Yeast	Acetic acid bacteria	Lactic acid bacteria	Heterotrophic bacteria
BL	50	-	5	> 300
AM	2	9	-	> 300
BA	> 300	9	2	-
CA	18	-	-	> 300
CN	> 300	-	-	-
OA	200	-	-	-

indicating that the degradation of lignin in these woods was satisfactory and directly influences the choice of wood for production as they increase the amount of volatile compounds and might cause an intensification of the color and quantity of organic acids, which gives the beer an aesthetic value. AM, CA and CN obtained between 7.05 and 7.71 %.

Relative density, alcoholic content and apparent extract

The relative density of the samples was measured by the Beer Analyzer densimeter and the results are shown in Table 2. Overall, the values obtained in wood-aged beer were similar to the reference beer (BL), which indicates the presence of the wood did not interfere the density, alcoholic content, primitive or apparent extract. Wyler et al. (2015) had similar results using oak cubes with different roasting temperatures and maturation of 90 days at 0°C.

pH, total acidity and microbiological analyses

The pH of the wood-aged beers had no significant variation ($p=0.05$) amongst them or with BL as shown in Table 3. The highest total acidity was from OA sample (0.802) and different from BL, 0.704 ($p=0.05$), indicating that the hemicellulose and lignin content of the wood influenced the oak acidity value. The total acidity was lower than Wyler et al. (2015) obtained with different oak toasting temperatures ($p=0.05$). The total acidity of other woods did not have a significant difference from BL ($p=0.05$). The microbiological analysis (Table 4) suggests that the presence of yeast inhibits the growth of heterotrophic bacteria; in all cases few or no lactic and acetic bacteria were found.

Colour, turbidity, and total phenolic compounds

The addition of wood to beer did not differ from reference beer (18.1 EBC, $p=0.05$), same results was obtained by

Table 5. Colour, turbidity and total polyphenol in beer.

Sample	Colour (EBC)	Turbidity (NTU)	Polyphenols (mg/L)
BL	18.1	54.30	107.0
AM	17.5	49.10	185.6
BA	18.1	49.72	167.4
CA	18.1	49.72	167.4
CN	18.4	55.62	170.8
OA	18.0	55.13	135.3

Wylter et al. (2015) with oak-aged beer for 90 days. The beer aged in presence of amburana (AM), balsam (BA) and Cariniana (CA) showed the lowest values of turbidity (Table 5), even lower than BL, which may be due to the adsorptive capacity and porosity from the woods used. Although the chestnut (CN) sample had a value close to AM and CA with regard to extractive content, it had a higher turbidity.

Comparing the wood-aged beer with BL, aged beer had an increase in total phenolic content, demonstrating that wood adds polyphenols to beer (WYLER et al., 2015), as also verified by the sensorial analysis. Beer aged with oak had a similar result to that of heavy toasted oak by Wylter et al. (2015). The AM sample was the one with the highest aroma and flavour intensity. Moreover, it was the one with highest polyphenol content (185.6 mg/L). At the end of the third month, CA and CN samples showed high polyphenols values (167.4 and 170.8, respectively), but aroma or flavour intensity were not as high.

HPLC

As stated above, wood aging is a technique used most for spirits and wines, although wood-aged beer are becoming more common. It is known that most molecules of interest in this technique are phenolic compounds. Some of them were measured by HPLC and the results are shown in Table 6. Wood aging increased significantly ($p=0.05$) the level of six phenolic molecules measured in all samples compared to the reference. They are syringaldehyde, syringic acid, synaldehyde, vanillin, vanillic acid and gallic acid. The low value of organic acids in all samples indicates that the contamination did not interfere with the results.

The results obtained for gallic acid, vanillin, vanillic acid and syringaldehyde in oak (OA) were similar (22.87, 1.02, 2.03 and 2.92 mg/L, $p=0.05$) to that previously reported by Wylter et al. (2015), whereas 5-HMF and synaldehyde were inferior, furfural and syringic acid were superior. Amburana-aged beer had the highest concentration of synaldehyde (2.87 mg/L) and 5-HMF. BA had the highest values for vanillin and vanillic acid (4.52 and 8.45 mg/L, respectively) and the lowest concentration of galic acid amongst the woods (8.01).

CA obtained the highest value for syringaldehyde (3.04 mg/L) and high values for syringic acid (6.57), vanillin and vanillic acid (3.82 and 6.92, respectively), gallic acid (20.33). The higher values of vanillin and vanillic acid in both BA and CA are in accordance with the sensorial analysis that identified a strong vanilla aroma. Chestnut-aged beer (CN) had the highest concentration of syringic acid (6.92 mg/L), coniferaldehyde (1.19) and furfural (4.28) amongst the woods studied. Even though oak is the most studied and used wood for aging and had a high extractable content, the Brazilian woods presented in this paper outdone it for several molecules, including vanillin and vanillic acid, which are responsible for the main aromas and flavour it offers.

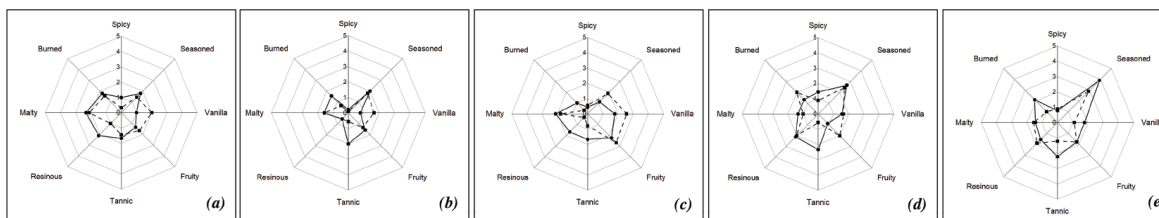
Zacaroni et al. (2011) in their paper about phenolic composition in cachaça production and aging in amburana, balsam, Cariniana and oak casks, observed the predominance of several different phenolic compounds. In chestnut cask aged cachaça, there was a predominance of gallic (12.14 mg/L) and ellagic (10.70 mg/L) acids. In oak, syringaldehyde (0.95 mg/L) and ellagic acid (2.08 mg/L); in amburana, coumarin (11.10 mg/L) and eugenol (6.69 mg/L); in balsam, syringic acid (0.40 mg/L) and ellagic acid (0.42 mg/L); in Cariniana, gallic acid (1.28-1.62 mg/L) and gallic acid (1.61-1.82 mg/L) (12). Santiago et al. (2014) observed only gallic acid (0.27 mg/L) and syringic acid (0.135 mg/L) in cachaça aged for 3 months in oak barrel. For the same time, amburana barrel presented gallic acid (0.51 mg/L), syringic acid (0.79 mg/L), along with several others polyphenols: vanillic acid (4.09 mg/L), syringaldehyde (0.81 mg/L) and sinapic acid (0.63 mg/L) to name a few.

Sensorial analysis

After 90 days of wood aging, flavour and aromas characteristics commonly associated with spirits wood aged were detected by the sensorial panel, as shown in Figure 2. Beer aged in the presence of wood not only showed higher levels of phenolic compounds, but also developed flavours associated with them, such as burned, condimented, vanilla, tannic and resinous, which were not detected in the reference beer. Most wood did not interfere with the malty flavour present in the reference

Table 6. Concentration of polyphenols and organic acids (mg/L) in beer aged in absence of wood (BL), and with amburana (AM), balsam (BA), Cariniana (CA), chestnut (CN), and oak (OA) cubes at 0°C by HPLC.

Sample	BL	AM	BA	CA	CN	OA
Syringaldehyde	0.93	2.76	2.23	3.04	2.76	2.92
Syringic acid	0.16	4.65	4.03	6.57	6.92	5.03
Sinapaldehyde	0.09	2.87	1.32	0.97	1.23	1.77
Coniferaldehyde	0.09	0.65	0.12	0.17	1.19	1.03
Vanillin	0.17	2.33	4.52	3.82	2.14	1.02
Vanillic acid	0.42	2.82	8.45	6.92	4.92	2.03
Genistein	0.07	0.18	0.05	0.17	0.22	0.10
Ellagic acid	0.02	0.23	0.09	0.19	0.17	0.31
Gallic acid	2.80	15.03	8.01	20.33	14.35	22.87
Caprylic acid	0.03	0.12	0.02	0.05	0.07	0.13
Guaiacol	0.01	0.18	0.03	0.18	0.21	0.14
5 – HMF	1.54	3.17	2.08	0.85	3.13	1.92
Furfural	1.82	1.98	2.93	1.18	4.28	2.84
Lactic acid	0.18	0.18	0.20	0.18	0.12	0.11
Acetic acid	0.05	0.18	0.09	0.03	0.01	0.10

**Figure 2.** Spider web plots of scores (0-5) on aroma (dashed) and flavour (line) descriptors after sensory analysis of wood-aged beers for Cariniana (a), chestnut (b), balsam (c), oak (d) and amburana (e). Reference beer without wood (BL) was rated 1 for malty and zero for others descriptors.

beer with the exception of oak and amburana, but both woods were rated as most aromatic by the panellists suggesting the malty aroma was hidden amongst the others. There was no report of any off-flavour in this analysis.

The aroma and flavour of amburana (AM), chestnut (CN) and oak (OA) were described as condimented or seasoned for their main aroma. CN had also a vanilla aroma, its strongest taste however, was burned, followed by condimented. This indicates there is no direct association between aroma and flavour. OA had also resinous and burnt as major aromas and a fainter vanillin one. Its taste was reported as resinous, tannic and condimented. It was the wood with the widest range of aromas and flavours. Cariniana (CA) and balsam (BA) had strong vanillin aromas. BA also developed a strong fruitiness aroma and its taste was closely related to its aroma, only slightly more resinous and less fruity.

Although the sensory analysis and the phenol concentrations measured by HPLC may not have an intricate relation, some molecules are known to contribute to some of the aromas evaluated. Wyler et al. (2015)

observed a relation between vanillin and vanillic acid and the vanilla aroma and also fruitiness aroma and catechin and scopoletin levels in cachaça. Lentz (2018) in his paper on phenolic compounds and their impact on aroma and flavour in beer cited guaiacol as one of the molecules responsible for the smoked aroma. In light of the data observed through sensorial analysis and HPLC results, Figure 3 was developed, which illustrates the molecules and their aroma or flavours found from the characteristic constituent of the composition of the woods studied herein.

Conclusion

The beer maturation process in presence of wood increases the sensorial and chemical complexity of it. The use of wooden cubes rather than barrels is an interesting alternative due to its practicality and low-cost process when compared to the use of barrels in its traditional way. The panellists identified different aroma and flavour profiles in the beer aged with different woods,

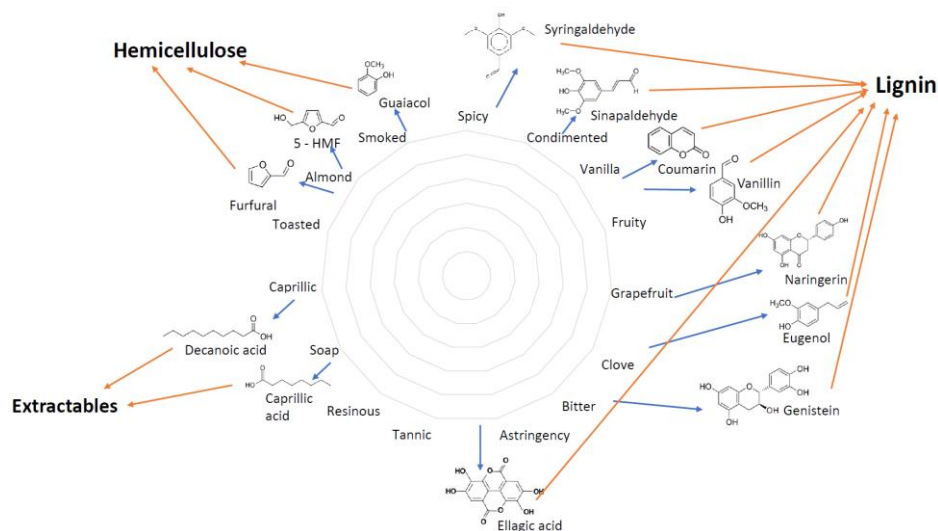


Figure 3. Sensorial profiles found in the beer samples.

indicating the wood interferes with the beer characteristics. Cariniana presented a strong vanilla aroma, which was also reported for balsam and chestnut. Amburana obtained the highest value for aroma and flavour and presented a more condimented aroma and resinous taste, similar sensations were reported for oak.

Overall, Brazilian wood showed to be readily available for this use and outdoes oak for many molecules, specially vanillin and vanillic acid. Yet more research needs to be done in both wood-aged beer and chemical and biochemical characterisation of Brazilian flora.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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