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Physicochemical characterization and volatile profile of liqueurs of Cerrado Brazilian fruits

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INFO

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ABSTRACT

The rich Brazilian biodiversity, especially native fruits with high nutritional value, such as genipapo and murici, have great economic potential and stand out in the manufacture of artisanal liqueurs. The objective of this work was to carry out the physical-chemical characterization and evaluate the volatile profile of fruit liquors from the Brazilian cerrado. Physicochemical analyzes were carried out, obtaining the following values for the genipapo liquor: pH 3.18; acidity 82.95 mg in acetic acid; density 1.13 g/L, dry residue 42.16 % m/v and °Brix 36.1. For the murici liqueur, the data obtained were pH 3.79; acidity 1.71 mg in acetic acid, density 1.09 g/L, dry residue 33.03% m/v and °Brix 36. The extraction of volatile compounds was also carried out using the solid phase microextraction technique in headspace mode (HS-SPME) and identification using the Gas Chromatography Coupled to Mass Spectrometry (GC-MS) technique, to characterize the volatile chemical profile of the drinks. The chemical classes found in the genipapo liquor were: carboxylic acids (69.6%), terpenoids 10.1%; alcohols, 8.7%; esters, 6.6%; aldehydes and ketones, 1.0%. The main compounds found in the aroma of genipapo liquor were hexanoic acid, octanoic acid, tetradecanoic acid, linalool and limonene. Esters were the most abundant in murici liquor (37.5%), followed by amines (11.99%), alcohols (8.57%), and ketones (0.18%). As a result, the liqueurs met quality standards.

Palavras-chaves
frutas nativas
jenipapo
murici
HS-SPME/GC-MS
bebida

RESUMO

Caracterização físico-química e perfil volátil dos licores de frutos do Cerrado Brasileiro
A rica biodiversidade brasileira, em especial as frutas nativas de alto valor nutricional, como o jenipapo e o murici, apresentam grande potencial econômico e se destacam na fabricação de licores artesanais. O objetivo deste trabalho foi realizar a caracterização físico-química e avaliar o perfil volátil de licores de frutas do cerrado brasileiro. Foram realizadas análises físico-químicas, obtendo-se os seguintes valores para o licor de jenipapo: pH 3,18; acidez 82,95 mg em ácido acético; densidade 1,13 g/L, resíduo seco 42,16 % m/v e °Brix 36,1. Já para o licor de murici os dados obtidos foram pH 3,79; acidez 1,71 g em ácido acético, densidade 1,09 g/L, resíduo seco 33,03 % m/v e °Brix 36. Realizou-se ainda a extração dos compostos voláteis através da técnica de microextração em fase sólida no modo headspace (HS-SPME) e a identificação através da técnica de Cromatografia Gasosa Acoplada à Espectrometria de Massas (GC-MS), para a caracterização do perfil químico volátil das bebidas. As classes químicas encontradas no licor do jenipapo foram: ácidos carboxílicos (69,6%), terpenóides 10,1%; álcoois, 8,7%; ésteres, 6,6%; aldeídos e cetonas, 1,0%. Os compostos majoritários encontrados no aroma do licor de jenipapo foram ácido hexanóico, ácido octanóico, ácido tetradecanóico, linalol e limoneno. Os ésteres foram os mais abundantes no licor de murici (37,5%), seguido de aminas (11,99%), álcoois (8,57%), e cetonas (0,18%). Com isso, os licores atenderam aos padrões de qualidade.

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INTRODUCTION

Brazil has a rich biodiversity and is known for its high-nutritional-value native fruits, which play an important role in the survival of indigenous populations (Assis et al., 2023; Hamacek et al., 2013). The use of native fruits adds commercial value to the fruit, generating income for rural families. But not just the commercial value, but also the social, historical and cultural value of those who work with it.

In the Cerrado and Amazon biomes, several native species can be found. The climatic variability and soil characteristics favor fruit growing in these biomes (Santos et al., 2023). Fruit species such as jenipapo (*Genipa americana* L.) (Claro et al., 2023) and murici (*Byrsonima crassifolia*) (Vinhal et al., 2022) are typical fruits found in these regions.

Jenipapo is a native Amazonian fruit but can be found in the cerrado due to its wide geographic distribution (Pacheco et al., 2014). Murici can be found in almost all Brazilian regions, such as the states of Mato Grosso and the North and Northeast regions of Brazil. They are used to manufacture various products, such as juices, nectars, jams, candies, ice cream, and as a filling in various products. They are also used in alcoholic beverage manufacturing, such as wines, artisanal cachaças, and liqueurs.

High perishability and storage problems lead to a scenario of great fruit waste, and processing is an alternative to avoid or reduce loss. To avoid these issues, liquor production is an option (Silva et al., 2021). Liqueurs are highly flavorful beverages with digestive, stimulant, and reconstituting properties and are homemade or industrially produced in several regions of the world (Roberto et al., 2020).

According to the Brazilian Legislation of the Ministry of Agriculture, Livestock, and Supply liqueur is a beverage with an alcoholic strength of 15 to 54% by volume, at 20 °C, with a sugar percentage above 30 g/L, made with an alcoholic part and a non-alcoholic part of vegetable or animal origin (Brasil, 2009).

According to Souza et al. (2019), fruits physicochemical characterization is important to know their nutritional value and, from the commercial point of view, to add value and quality to the final product and the profile of compounds found may have a viable utilization as it presents industrial potential for the elaboration of liqueurs. The aroma of the liqueur is highly dependent on the qualitative and quantitative composition of volatile compounds, one of the most important attributes that affect beverage consumption (Alves, 2004).

The modern analysis technologies afford to achieve an extensive knowledge of the volatile composition of the pulps and fruit liquors, by increasing substantially the number of compounds identified in the Brazilian cerrado biome. The objective of the work was to carry out the physical-chemical characterization and evaluate the volatile profile of fruit liquors from the Brazilian cerrado.

MATERIAL AND METHODS

Obtaining the samples

The samples of murici liqueur and jenipapo liqueur were purchased from the Cocada Doces & Biscoitos micro-enterprise located in the city of Porto Nacional-TO. The samples were stored away from light and heat to avoid alterations in their properties, at room temperature, until the analyses were performed.

Physicochemical analysis of craft liqueurs from murici and jenipapo

The methodology used to perform the physical-chemical experiments was based on the Adolfo Lutz Institute Manual (2008). All analyses were performed in triplicate.

pH determination

To determine the pH, a pHmeter (Thermo Scientific, Model: Orion Star A211) previously calibrated with pH 4, 7, and 14 buffer solutions was used. The instrument's sensor was immersed in the liquor samples for pH measurement.

Determination of titratable acidity

To determine the total titratable acidity, an aliquot of each liquor sample was transferred separately to an erlenmeyer flask, and three drops of the phenolphthalein indicator were added. The 50 mL burette was filled with the standardized 0.1 mol/L NaOH solution, according with (IAL, 2008). All analyses were performed in triplicate.

Relative density at 20 °C

To analyze the relative density of the liqueurs, a glass pycnometer previously washed according to the following sequence was used: distilled water, alcohol, and ether. It was then dried at room temperature and weighed on an analytical scale. Next, a 10 mL aliquot of distilled water was transferred at a temperature of 20 °C to the pycnometer, which was weighed again. Afterwards, the pycnometer was rinsed with distilled water, alcohol, and ether, respectively, and then dried in the open air at room temperature. The same procedure was performed with the liqueur samples. The relative density was performed according with (IAL, 2008).

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Dry residue

To analyze the dry residue, a 10 mL aliquot of the liqueur samples was transferred to a porcelain crucible and dried in a water bath at a temperature of 100 °C. The samples were heated until the water and alcohol evaporated totally for a period of 3 hours and 30 minutes.

Then, all the material was taken to dry in an oven (Medclave, Model 3) at 105 °C for 30 minutes and, after cooling, it was weighed on a calibrated analytical scale to obtain the dry residue of the sample, according with (IAL, 2008).

Soluble solids (°Brix)

For the analysis of soluble solids, a refractometer (Salvi Casagrande, Model OD. 107 Kiltler, NF: 32362) was used at a temperature of 20 °C, where it was previously calibrated with distilled water. Then, an aliquot of the liqueur samples was added to the apparatus and read in degrees of °Brix. For this analysis, a 0 to 90% °Brix Refractometer was used.

HS-SPME/GC-MS chromatographic analysis

For the chromatographic analysis, the methodology used was based on Śliwińska et al. (2016) with modifications changes in the temperature ramp of the chromatograph oven, which used headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) to analyze volatile compounds in artisanal liqueurs produced from local fruits. To perform the experimental procedures, the methodology was adapted for HS-SPME/GC-MS.

For this, a 10 mL aliquot of the beverage was transferred to a 15 mL vial with a silicone cap, which was heated to 40 ºC for approximately 15 minutes in a magnetic stirrer/heater. Then the SPME fiber was exposed to the sample headspace, remaining under heating for another 15 minutes for extraction of the volatiles. Then, the fiber was retracted and inserted into a GC-MS system model GC-7890B coupled to a mass spectrometer model MS-5977B by Agilent Technologies. The chromatographic column used was a fused silica capillary type, with a stationary phase of HP-5 MS 5% phenyl methyl siloxane, with the following dimensions: 30 m (length) x 25 µm (external diameter) x 0.25 µm (internal diameter). The carrier gas used was high purity helium 5.0. The temperature programming was an initial oven temperature equal to 50 ºC remaining for two minutes, followed by a rate of 6 ºC/min until reaching 170 ºC, followed by another rate of 25 ºC/min until reaching 250 ºC, remaining for three minutes. The temperature of the detector and analyzer were 250 ºC and 150 ºC, respectively. The injected sample volume was 0.5 µL in Splitless manual mode.

RESULTS AND DISCUSSION

Physical-chemical analysis of the liquors

Table 01 shows the values obtained from the main physical-chemical analyses for the liqueur samples. The pH values found in the liqueurs were 3.18 for the jenipapo liqueur and 3.79 for the murici liqueur. Some close values found by Carlos et al. (2017) in murici pulps with pH from 3.20 to 3.70 and by Silva et al. (1998) for stored jenipapo fruit with pH of 3.05 corroborate the values obtained in this study. Silva et al. (1998) observed that the fruit with zero days of storage had a higher pH than the fruit with 28 days of storage, concluding that the fruit has a very acid pH, contributing to the drop of pH in the liqueur.

Table 01 - Physicochemical analysis of Jenipapo and Murici liqueurs

Components	*Jenipapo liqueur*	*Murici liqueur*
pH	3.18±0.03	3.79±0.02
Titratable total acidity	82.95±10.4	1.71±0.04
Density	1.13±0.01	1.09±0.01
Dry residue % (m/v)	42.16±3.8	33.03±4.53
Soluble solids (°Brix)	36.1±2.5	36±2.83

The total acidity obtained in the liqueurs was 82.95 mg of acetic acid in 100 mL of sample of the jenipapo liqueur and 1.71 g of acetic acid in 100 mL of sample of the murici liqueur. No similar works were found with studies on jenipapo liqueurs to compare the values obtained in this research. Carlos et al. (2017) obtained a mean value for titratable acidity of 1.79 of the murici pulp, a value close to that found in this study.

For the analyses of density, dry residue, and soluble solids (ºBrix), the legislation does not determine fixed values; however, it is understood that the lower their values, the better the quality of the Jenipapo water and liquor.

In the samples analyzed, a density of 1.13 g/L was found in the jenipapo liqueur and 1.09 g/mL in

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the murici liqueur. Compared to the jenipapo liqueur, the density analysis of the Cachaça 51 used in the beverage preparation found a density of 0.95 g/L. Thus, the jenipapo liqueur it is of good quality as required by law. The study by Santos et al. (2018) used a similar experimental approach and determined a density of 1.07 g/mL for murici pulp.

It was also established that the average value of dry residue was 42.16% m/v of residue for the jenipapo liqueur and 33.03% w/v of residue for the murici liqueur. The residue value found for the jenipapo liqueur is acceptable due to the addition of sucrose in its composition. The study conducted by Nascimento et al. (2016) for pineapple peel liqueur in different concentrations of mint obtained values ranging from 35.08 to 36.52% residue, values very close to those found in murici liqueur.

For soluble solid content (ºBrix), the Jenipapo liqueur averaged 36.1 and the Murici liqueur 36.2. Hansen et al. (2008) found a value of soluble solids for the jenipapo fruit in the state of Bahia with a ºBrix of 23.46, a value below that obtained in this study for the jenipapo liqueur, which can be explained by climatic factors and soil type, which strongly influence the fruit's characteristics. In the analyses performed by Nascimento et al. (2016) on pineapple liqueur with different mint concentrations, the values of soluble solids were similar to those found in this study for murici liqueur, with a ºBrix value of 36.

Volatile constitution of liqueurs

Jenipapo liqueur

Table 02 shows the compounds identified in the jenipapo liquor, with 24 compounds identified by the mass spectrometer NIST/2014 library (National Institute of Standards and Tecnology) and the Kovats retention index (IR).

The main organic functions of the compounds identified were hydrocarbon, alcohol, terpene, acid, and ester. These data agree with previous work reporting the presence of these classes of compounds in the headspace of the jenipapo and in the aqueous solution of the fruit (Pinto et al., 2006; Borges and Rezende 2000).

Table 2 - Volatile compounds identified by GC-MS in the fine Jenipapo liqueur

Chemical compound	Retention time (min.)	Experimental RI	RI reference (NIST)
	Hydrocarbon		
Styrene	5.559	893	900
	Alcohol		
3-Methyl-Butanol Alcohol	2.851	732	—
	Terpene		
Nerolidol	20.779	1564	1566
	Acids		
Octanoic Acid	13.384	1180	1221
	Esters		
Ethyl Acetate	2.071	612	—
Methyl 2-methylbutanoate	4.759	849	—
Isoamyl Acetate	5.213	876	—
Methyl Hexanoate	6.174	925	924
Ethyl Hexanoate	8.041	1000	1002
Methyl Heptanoate	8.636	1023	1027
Ethyl Cyclohexanoate	10.195	1136	1090
Ethyl Heptanoate	10.451	1097	1100
Methyl Octanoate	11.097	1126	1126
Ethyl Octanoate	13.087	1196	1209
Propyl Octanoate	15.043	1290	1293
Ethyl Nonanoate	15.118	1269	1297
Methyl Decanoate	15.754	1325	1326
n-Caprylic Acid Isobutyl Ester	16.277	1348	1350
Ethyl 9-decenate	17.149	1387	1391
Ethyl Decanoate	17.333	1396	1400
Isopentyl Octanoate	18.379	1446	1448
Octanoate-2-Methyl Butyl	18.451	1449	1452
Ethyl 3-phenyl-2-propenoate	18.810	1463	1469
Ethyl Dodecanoate	21.354	1595	1595

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The esters ethyl acetate and isoamyl acetate were found in the headspace of the jenipapo liqueur for the first time. Methyl 2-methylbutanoate was also found in the liqueur and was cited by Pinto et al. (2006) as a potent volatile constituent, which imparts fruity characteristic and candies notes to the jenipapo. In addition to these, methyl hexanoate and methyl octanoate esters were found in the headspace of the liqueur and were noted as odorants with green and candies fruity notes of the jenipapo aroma by Pinto et al. (2006), in work where groups of odorous compounds in headspace and total aqueous extracts of jenipapo were determined by GC-MS and aroma extration dilution analysis gas chromatography-olfactometry (AEDA/GC-O).

Pino et al. (2005) determined the volatile constituents in the jenipapo fruit from Cuba. Eighty-one of these constituents were reported for the first time in the fruit. The chemical classes reported were carboxylic acids, comprising the largest class of volatiles (69.6%). The composition of the other classes was terpenoids, 10.1%; alcohols, 8.7%; esters, 6.6%; aldehydes and ketones, 1.0%; and others, 3.9%. The major compounds found in the jenipapo aroma were hexanoic acid, octanoic acid, tetradecanoic acid, linalool, and limonene.

According to Franco and Janzantti (2005), jenipapo has a high content of carboxylic acids, butanoic, 2-methylbutanoic, and hexanoic acids, and volatile compounds responsible for the acid notes. Moreover, the ethyl esters of 2- and 3-methylbutanoic acids showed intense and characteristic notes of the fruit, as determined by GC-Olfactometry.

In this study, the compounds found in the jenipapo liqueur, which are common to the fruit, are octanoic acid, methyl hexanoate, ethyl hexanoate, methyl heptanoate, methyl octanoate, and ethyl octanoate. Moreover, we also identified the presence of the terpenoid nerolidol for the first time.

Murici liqueur

Table 03 shows the compounds identified in the murici liquor. Thirteen volatile compounds were detected, eight positively identified, and five tentatively identified.

The main volatile compounds found in the beverage were propyl hexanoate, 2-methylpropyl hexanoate, diethyl butanedioate, ethyl octanoate, 2-methylbutyl hexanoate, 3-methylbutyl octanoate and ethyl decanoate. The compounds ethanol, 3-methylbutan-1-ol, methylhydrazine, ethyl acetate, and ethyl butanoate were also identified, but could not be verified using the Korvat's Index.

Table 3 - Volatile compounds identified by GC-MS in Murici liqueur

Chemical compound	Retention time (min.)	Experimental RI	RI reference (NIST)
	Alcohol		
Ethanol	1.836	NI	427
3-Methylbutan-1-ol	2.872	NI	736
	Amine		
Methylhydrazine	1.580	NI	—
	Ketone		
(E)-hept-3-en-2-one	6.574	1012	—
	Esters		
Ethyl Acetate	2.020	NI	612
Ethyl Butanoate	3.825	NI	802
Propyl Hexanoate	10.359	1098	1094
2-Methylpropyl Hexanoate	11.754	1153	1149
Diethyl Butanedioate	12.472	1183	1184
Ethyl Octanoate	13.025	1201	1196
2-Methylbutyl Hexanoate	14.143	1255	1247
3-Methylbutyl Octanoate	18.390	1450	1446
Ethyl Decanoate	17.333	1400	1396

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Uekane et al. (2017), reported in a study on the identification of volatile compounds in some Amazonian fruits, including murici, esters are also more abundant in murici liquor (38%), similar the results shown in Table 03. The predominant and majority compounds in this work are esters (37.5%), followed by amines (11.99%), alcohols (8.57%), and ketones (0.18%).

Hexanoate and octanoate were predominant among the esters. These compounds may favor less specific interactions between other compounds present in the matrix, facilitating their volatility in systems with low energy and at ambient conditions. Therefore these esters may be responsible for the typical aroma of this fruit in the murici liqueur in this study, since no isolated compound showed the characteristic aroma of the fruit (Uekane et al., 2017).

As a result, alcohols such as ethanol were found, as they originate from the alcoholic matrix used in the production process of several non-fermented liqueurs; and 3-methylbutan-1-ol (isoamyl alcohol), which can be produced by low-energy reaction processes and/or be the result of other processes related to fruit degradation. As ascertained in this study, (Almeida and Barreto, 1971) analyzed a sugarcane matrix and determined isoamyl alcohol, which is also a predominant compound in several alcoholic beverages, such as wines, beers (Gomes et al., 2014), and spirits (Nóbrega, 2003).

Then, as Table 03, methylhydrazine (amine) presents itself as the second majority compound before the alcohols. Thus, this compound is a derivative of hydrazine, as reported in the work of Gomes et al. (2014). However, the authors point out that the presence of amines in low concentrations affects the quality of various food products. Consequently, amines influence negatively and directly on the sensitive quality of beverages such as beers and wines.

Another class of compounds found in the liqueur is (E)-hept-3-en-2-one, which is a ketone compound present in a lower percentage area among the major compounds in this study. In the work of Azevêdo et al. (2007), some ketone compounds in wines were determined. In a study directed at wine quality, they showed that the ketone compounds are the result of metabolites present in the different stages of production of these beverages.

Azevêdo et al. (2007) reported that such compounds account for the characteristic and pleasant aroma in some types of wines, distinguishing them positively over undesirable odors and flavors from compounds in high concentrations of esters, alcohols, and aldehydes. Alves (2004) studied the volatile compounds present in murici, using an experimental procedure similar to the one employed in this work, detected 51 volatile compounds, 41 of them identified and five considered tentatively identified. The most abundant chemical classes were esters (54-56%), followed by alcohols (36-27%).

In a final approach on compounds present in murici fruit, Rezende and Fraga (2003) studied the volatile compounds of murici from the north Brazilian region (Belém-PA) and detected 95 substances, of which 50% are esters, 10.4% ketones and aldehydes, 9.4% carboxylic acids, 8.3% terpenoids, 6.2% alcohols, 4.1% lactones, 4.1% sulfur compounds, and 7.6% other substances. Thus, the unique differences found in this study and those reported in the studies by Alves (2004) and Rezende and Fraga (2003) may refer to the different isolation techniques used in the liqueur analysis, since infusing the fruit in alcohol for a long time may favor the broad spectrum observed.

CONCLUSIONS

The jenipapo and murici liqueurs presented physical-chemical characteristics in accordance with the standards required by Brazilian legislation and related literature. There are no fixed values established by law for these analyses; however, they are of utmost importance as they prevent the appearance of deteriorating substances, thus ensuring the quality of the liqueurs.

The esteres was the present compost in murici fruits, and carboxylic acids in jenipapo liqueurs. The chromatographic analyses are important resources in the characterization and monitoring of the chemical and volatile constitution of beverages. These liqueurs derived from simple technologies show potential to adding value to these national fruits.

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