

Fermented beverages based on *Hylocereus lemairei* (Hook.) fruits: Chemical characterization and antioxidant capacity evaluation

Bebidas fermentadas à base de frutas *Hylocereus lemairei* (Hook.): caracterização química e avaliação da capacidade antioxidante

Bebidas de frutas fermentadas *Hylocereus lemairei* (Hook.): caracterización química y evaluación de capacidad antioxidante

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Abstract

Hylocereus lemairei (Hook.) is a plant popularly known as pitaya-purple, whose fruits are known as dragon fruit. This paper aimed to carry out the physical-chemical and chemical characterization of fermented beverages based on *H. lemairei* fruits. Fermented beverages were elaborated using two different commercial yeasts of *Saccharomyces cerevisiae* [Biolievito Bayanus (BB) and Arom Cuvée (AC)] and were characterized by NMR. In addition, the antioxidant capacity (DPPH and ABTS) and Total Phenolic Content (TPC) based on the Folin Ciocalteu method were determined. According to the legislation, the fermented beverages from BB and AC showed alcoholic levels of 12.9 and 12.5% (v/v) and pH of 3.9 and 3.8, respectively. The chemical compounds of both beverages were similar, whose major organic compounds are glycerol, myo-inositol, tyrosol, and citric and succinic acids. According to the DPPH and TPC evaluation, BB beverage (248.3 $\mu\text{M TE}$ and 117.6 mg GAE L⁻¹) presented higher antioxidant capacity when compared to the BC beverage (219.8 $\mu\text{M TE}$ and 108.4 mg GAE L⁻¹). In the ABTS assay, both beverages were not statistically different ($p < 0.05$). Tyrosol may be responsible for increasing the antioxidant capacity and phenolic compounds content when compared to the control juice used to prepare fermented beverages.

Keywords: Pitaia-roxa; Dragon fruit; Biolievito Bayanus; Arom Cuvée; Tyrosol.

Resumo

Hylocereus lemairei (Hook.) É uma planta popularmente conhecida como pitaia-roxa, cujos frutos são conhecidos como fruta-do-dragão. Este trabalho teve como objetivo realizar a caracterização físico-química e química de bebidas fermentadas à base de frutos de *H. lemairei*. As bebidas fermentadas foram elaboradas a partir de duas diferentes leveduras comerciais de *Saccharomyces cerevisiae* [Biolievito Bayanus (BB) e Arom Cuvée (AC)] e caracterizadas por RMN. Além disso, foram determinadas a capacidade antioxidante (DPPH e ABTS) e o Conteúdo de Fenólicos Totais (CFT) pelo método de Folin Ciocalteu. De acordo com a legislação, os fermentados de BB e BC apresentaram teores alcoólicos de 12,9 e 12,5% (v/v) e pH de 3,9 e 3,8, respectivamente. Os compostos químicos das bebidas elaboradas foram semelhantes, cujos principais compostos orgânicos são o glicerol, o mio-inositol, o tirosol e os ácidos cítrico e succínico. De acordo com a avaliação DPPH e CFT, a bebida BB (248,3 µM TE e 117,6 mg GAE L⁻¹) apresentou maior capacidade antioxidante quando comparada à bebida BC (219,8 µM TE e 108,4 mg GAE L⁻¹). No ensaio ABTS, essas bebidas não foram estatisticamente diferentes ($p < 0,05$). O tirosol pode ser responsável por aumentar a capacidade antioxidante e o conteúdo de compostos fenólicos em comparação com o suco controle usado para preparar bebidas fermentadas.

Palavras-chave: Pitaia-roxa; Fruta-do-dragão; Biolievito Bayanus; Arom Cuvée; Tirosol.

Resumen

Hylocereus lemairei (Hook.) es una planta conocida popularmente como pitaya-púrpura, cuyos frutos se conocen como fruta del dragón. Este trabajo tuvo como objetivo realizar la caracterización físico-química y química de bebidas fermentadas a base de frutos de *H. lemairei*. Las bebidas fermentadas fueron elaboradas utilizando dos levaduras comerciales diferentes de *Saccharomyces cerevisiae* [Biolievito Bayanus (BB) y Arom Cuvée (AC)] y se caracterizaron por RMN. Además, se determinó la capacidad antioxidante (DPPH y ABTS) y el Contenido Fenólico Total (TPC) por el método de Folin Ciocalteu. Según la legislación, las bebidas fermentadas de BB y BC presentaron parámetros físico-químicos con niveles alcohólicos de 12,9 y 12,5% (v/v) y pH de 3,9 y 3,8, respectivamente. Los compuestos químicos de las bebidas elaboradas fueron similares, cuyos principales compuestos orgánicos son glicerol, mioinositol, tirosol, ácidos cítrico y succínico. Según la evaluación de DPPH y TPC, la bebida BB (248,3 µM TE y 117,6 mg GAE L⁻¹) fue más alta en comparación con la bebida BC (219,8 µM TE y 108,4 mg GAE L⁻¹). En el ensayo ABTS, estas bebidas no fueron estadísticamente diferentes ($p < 0,05$). El tirosol puede ser responsable de aumentar la capacidad antioxidante y el contenido de compuestos fenólicos en comparación con el jugo utilizado para preparar bebidas fermentadas.

Palabras clave: Pitaia-púrpura; Fruta del dragón; Biolievito Bayanus; Arom Cuvée; Tirosol.

1. Introduction

Fermented fruit is defined as a beverage with an alcoholic degree from 4 to 14% (v/v, 20 °C). Alcoholic fermentation can be obtained from fresh and ripe fruits of a single species, as well as from the respective integral juice, concentrate or pulp (Brasil, 2009). The process of obtaining fermented fruits is very similar to the wine production process with different operations according to the fruit type. Alcoholic fermentation is a relatively efficient preservation process because it increases the shelf life of a food product and reduces refrigeration or other forms of preservation technology. This technique is suitable in remote areas, where other preservation technologies are not available or inaccessible (Battcock & Azam-Ali, 1998).

The species from the family Cactaceae was possibly originated in North, Central and South America. The genus *Hylocereus* is native from the tropical deciduous forests in Mexico, Western India, Central America and northern South America. The species are known as pitaya, whose fruits are called dragon fruit in Asia. Pitaya enhanced the interest of the consumer because it is exotic and a source of vitamins and minerals. Interesting for fruit growers is mainly based on their high commercial value (Bastos et al., 2006; Fernandes et al., 2017; Nunes et al., 2014).

In order to add value to the fruit and elaborate a new beverage with attractive colors, the species *Hylocereus lemairei* (Hook.) Britton & Rose from the Cactaceae family was selected. Its fruits are popularly known as pitaya-purple, pitaya-red, pitaia, pitaya, pitahya roja or dragon fruit. It is a succulent epiphyte species, and have been cultivated in different regions of Brazil, including the Amazon region. Its fruits are globose, red, berry-like and without thorns with purple fleshy pulp and black seeds. It has the same water-soluble pigment found in beet, known as betalain. These *N*-heterocyclic compounds are categorized as betacyanins (red-purple color) and betaxanthines (yellow-orange color), which are natural antioxidants (Herbach; Stintzing; Carle, 2004; Kinupp; Lorenzi, 2014; Nunes et al., 2014).

This work aims to determine the physical-chemical parameters, as well as the chemical profiles of fermented beverages from *H. lemairei* fruits. These alcoholic beverages were prepared using two different commercial yeasts and the antioxidant capacity and total phenol content were determined.

2. Methodology

2.1 Preparation of fermented beverages

Hylocereus lemairei fruits were purchased at the Adolpho Lisboa Market (Manaus, Amazonas). Fruits were washed and sanitized with peracetic acid (0.5 g L^{-1}), cut and processed in a blender to obtain the juice. The yeast was prepared in a 1.0:1.4 ratio (fruit/water, m/v), chaptalized to 21 °Brix and the pH (3.5) was corrected using tartaric acid. Fermentation process were carried out in glass bottles equipped with an airlock valve and conducted at 20 °C by the commercial yeasts Biolievito Bayanus (BB) and Arom Cuvée (AC) (Biotecsul, Caxias do Sul).

The fermentation evolution was monitored at regular intervals by the Total Soluble Solids - TSS (°Brix). Racking was performed after fermentation at 20 °C, followed by vacuum filtration with diatomaceous earth. The fermentation was stored in glass bottles with synthetic stoppers (Boeira et al., 2020). The following physicochemical analyzes were carried out (density at 20 °C, pH, alcohol content, total sugars, total acidity, volatile acidity, ash, and reduced dry extract) (Jacobson, 2006; Rizzon, 2010).

2.2 ^1H NMR analysis of *Hylocereus lemairei* fermented beverages

A juice aliquot (550 μL) was mixed with 50 μL of D_2O (0.6 mM TMS- d_4). After ethanol removal in the absence of light, the fermented beverages were solubilized in 600 μL of D_2O (0.6 mM TMS- d_4). Samples were subjected to a Bruker® Avance IIIHD Nuclear Magnetic Resonance Spectrometer (11.74 T, BBFO Plus SmartProbe™) at 298 K. The ZGPR sequence was used for analyzing the samples and suppression of the residual water signal (4.8 ppm). The obtained spectra were processed by TopSpin™ 4.1.1 software. Chemical identification of the compounds was appropriately marked based on the chemical shift (δ_{H} and δ_{C}), coupling constant (J in Hertz), and comparison with literature data.

2.3 Evaluation antioxidant capacity and quantification of total phenols

2.3.1 Scavenging capacity of DPPH· and ABTS⁺ radicals

Juice and fermented beverage were subjected to the scavenging capacity of DPPH· and ABTS⁺ radicals and the determination of total phenols was based on methodologies with marginal modifications on a microplate reader (Molyneux, 2004; Re et al., 1999; Souza et al., 2020). For the DPPH assay, 10 μL of the sample was added to 190 μL of the DPPH solution (60 μM) and incubated in a dark environment for 30 min. Subsequently, the absorbances were read on a Microplate Reader at 515 nm (Epoch 2, Biotek). A standard Trolox curve from 100 to 500 μM was obtained ($y = -0.0007x + 0.5322$, $R^2 = 0.9999$). For the ABTS assay, the sample was mixed with the ABTS solution (1:100) and incubated in a dark environment for 6 min. Subsequently, the absorbances were read in the same microplate reader at 734 nm. A standard Trolox curve from 62.5 to 500 μM was made ($y = -0.0002x + 0.6374$, $R^2 = 0.9999$). Results were expressed in micromolar of Trolox Equivalents ($\mu\text{M TE}$). Measurements were performed in triplicate.

2.3.2 Total Phenolic Content (TPC)

For the TPC content evaluation, samples were added to the reaction mixture (1:1) of Folin Ciocalteu reagent and sodium bicarbonate (6 g L^{-1}), kept in the dark for 90 min for further analysis on a microplate reader at 725 nm (Epoch 2, Biotek). The standard curve for gallic acid was performed from 7.8 to 500 $\mu\text{g mL}^{-1}$ ($y = 0.0043x + 0.0289$, $R^2 = 0.9999$).

Results were expressed in milligrams of Gallic Acid Equivalents per liter of a sample (mg GAE L^{-1}) (Velioglu et al., 1998; Souza et al., 2020). This assay was performed in triplicate.

2.4 Statistical analysis

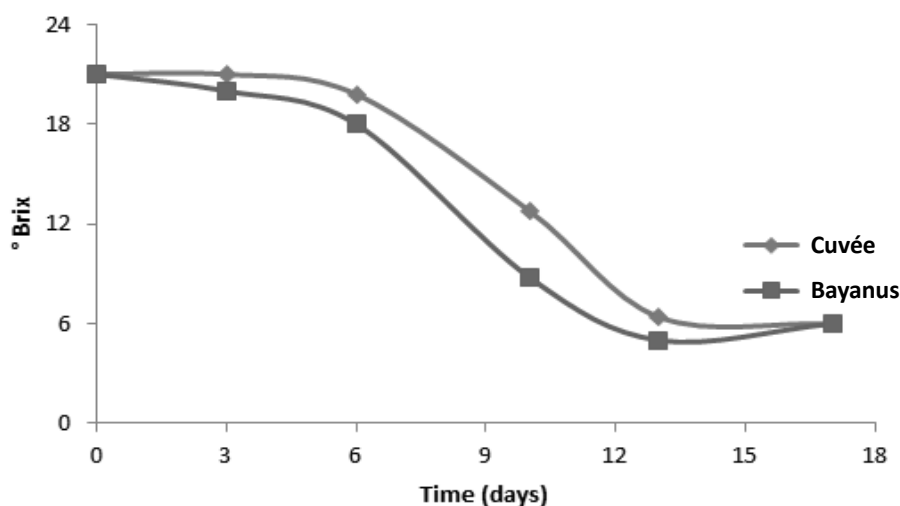
ANOVA (One-Way, Tukey's test, 95% significance) and Test-T ($p < 0.05$) to evaluate the assays. Results were expressed as mean \pm standard deviation. The Minitab® 18.1 software was employed in these analyses.

3. Results and Discussion

3.1 Physicochemical Analysis

The fermentation evolution was monitored by the content of TSS ($^{\circ}\text{Brix}$) over time regular intervals. The end of fermentation was determined when the TSS content stabilized on two consecutive measurements in 17 days with a TSS of 6 for both fermented beverages (Figure 1).

Figure 1. Alcoholic fermentation curves of *Hylocereus lemairei* fruits.



Source: Authors.

Then, fermented beverages from *H. lemairei* fruits were subjected to analysis of physical-chemical parameters (Table 1). The fermented products obtained from different yeasts presented an alcohol content within the range established by the current legislation from 4 to 14% v/v, with 12.9 and 12.5% (v/v) for the yeasts BB and AC, respectively. These densities were found to be similar among the fermented samples, 0.988 g mL^{-1} . The pH of the beverage was 3.8 to 3.9. The values of total acidity in the beverage fermented by the yeasts BB and AC were 63.6 mEq L^{-1} and 62.1 mEq L^{-1} , respectively. The acidity observed by current legislation for fermented fruit ranges from 50 mEq L^{-1} to 130 mEq L^{-1} , so both fermented are in accordance with the reference values. The volatile acidity values were 9.6 mEq L^{-1} for BB and 10.0 mEq L^{-1} for the AC beverage, which are in agreement to the standards established by the legislation for fruit fermented (maximum 20.0 mEq L^{-1}). The alcoholic beverages produced by the yeasts BB and AC showed dry extract content of 21.2 g L^{-1} and 20.7 g L^{-1} , respectively. The ash concentration found in the AC beverage (3.1 g L^{-1}) was slightly higher than that of BB 2.6 g L^{-1} . The average ashes content for fermented beverages was found from 1 to 3 g L^{-1} (Brasil, 2012).

Table 1. Results obtained for the physical-chemical parameters analyzed in the fermented beverages produced with *Hylocereus lemairei* fruits

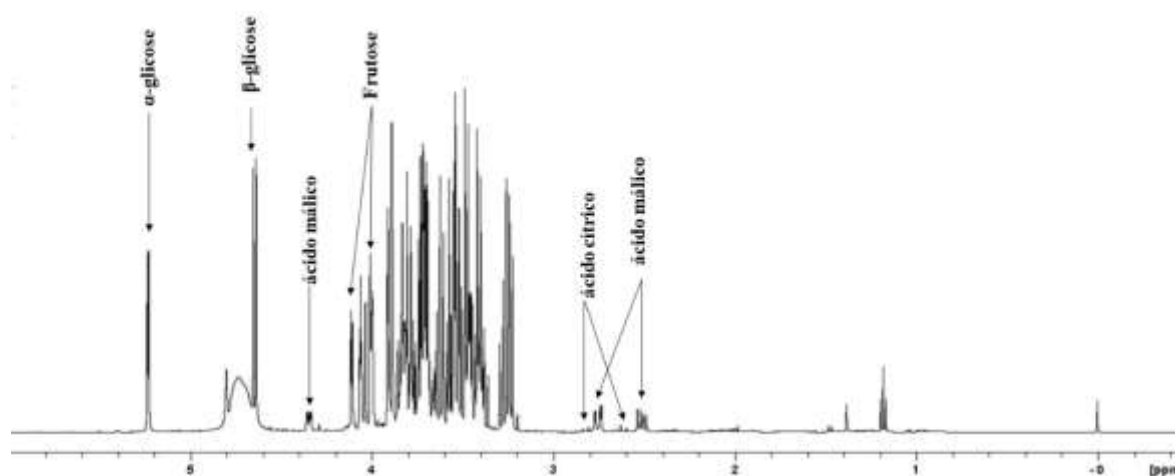
Sample	alcohol content (%)	density (g mL ⁻¹)	pH	total acidity (mEq L ⁻¹)	volatile acidity (mEq L ⁻¹)	dry extract (g L ⁻¹)	ash (g L ⁻¹)
BB	12.9 ± 0.1 ^a	0.99 ± 0.00 ^a	3.9 ± 0.0 ^a	63.6 ± 0.4 ^a	9.6 ± 0.4 ^a	21.2 ± 1.1 ^a	2.6 ± 0.1 ^b
AC	12.5 ± 0.2 ^a	0.99 ± 0.00 ^a	3.8 ± 0.0 ^a	62.1 ± 0.6 ^b	10.0 ± 0.4 ^a	20.7 ± 1.2 ^a	3.1 ± 0.3 ^a

Results are expressed as means ± standard deviation (n=3). ^{a-b}Same letter in the same column are statically significant (*p*-value < 0.05). Source: Authors.

3.2 ¹H NMR analysis of juices and fermented beverages

The major signs characteristic of carbohydrates and organic acids were observed from the ¹H NMR spectrum (Figure 2, Table 2). The signals at δ_H 5.23 (d, *J* = 3.8 Hz) and at δ_H 4.64 (d, *J* = 8.0 Hz) were attributed to α -glucose and β -glucose, respectively. The doublet in δ_H 4.11 (*J* = 3.7 Hz) and the multiplet in δ_H 3.99 were signed to fructose. The doublets at δ_H 2.82 and δ_H 2.61 (*J* = 16.0 Hz) were referred to the hydrogens of citric acid. The signals at δ_H 4.34 (dd, *J* = 8.8 and 3.7 Hz), δ_H 2.75 (dd, *J* = 15.7 and 3.7 Hz) and δ_H 2.51 (dd, *J* = 15.7 and 8.8 Hz) were attributed to malic acid. Data were compared with the scientific literature (Alves Filho et al., 2021).

Figure 2. ¹H NMR spectrum of *Hylocereus lemairei* juice (H₂O:D₂O, 11.74 T).



Source: Authors.

The analysis of chemical profiles by ¹H NMR of *H. lemairei* fermented beverage obtained with the BB and AC yeasts were similar (Figure 3, Table 2). The correlation maps HSQC and HMBC contributed to the confirmation of the compounds. The data were compared with the scientific literature (Al-Mekhlafi et al., 2021; Souza et al., 2020). After the fermentation process, no signs of sugars were observed, as there was a conversion to ethanol. After removed of ethanol, the analyzed extracts of both fermenters showed major signals of organic acids.

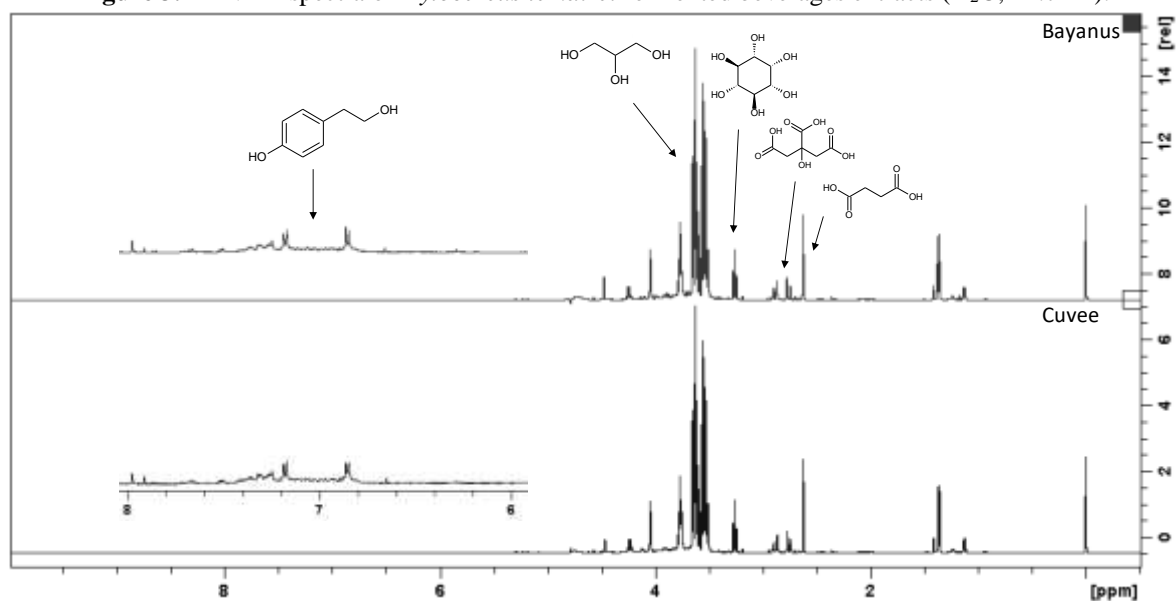
The signal at δ_H 3.54 (dd, *J* = 11.7 and 6.5 Hz) and at δ_H 3.64 (dd, *J* = 11.7 and 4.3 Hz) were directly linked to carbon at δ_C 65.5. The multiplet at δ_H 3.77 ppm linked to carbon at δ_C 75.0 ppm was attributed to glycerol. The signal at δ_H 4.05 ppm (t, *J* = 2.9 Hz) attached to carbon at δ_C 75.1, δ_H 3.61 (t, *J* = 9.5 Hz), δ_H 3.52 (dd, *J* = 9.5 and 2.9 Hz) and δ_H 3.26 (t, *J* = 9.5 Hz) was assigned to myo-inositol. The doublets at δ_H 2.76 and δ_H 2.88 (*J* = 15.6 Hz) linked to carbon in δ_C 46.7 was attributed to

citric acid. The signal at δ_H 2.62 (s) attached to carbon at δ_C 32.5 for succinic acid. Minority signals in the aromatic region, with the doublets in δ_H 7.18 (d, $J = 8.5$ Hz) and δ_H 6.86 (d, $J = 8.5$ Hz) linked to the respective carbons in δ_C 133.4 and δ_C 118.4 were attributed to tyrosol.

The compounds glycerol, citric acid, and succinic acid were also found in the fermented beverage from dragon fruit (*H. costaricensis*). Higher glycerol content was observed in the fermentation with *Saccharomyces cerevisiae* when compared to different yeasts (Jiang et al., 2020). Glycerol is produced as a by-product of *S. cerevisiae* ethanolic fermentation process and is responsible for the smoothness and viscosity of wine (Scanes et al., 1998). Inositol has previously been identified in dragon fruit varieties (Al-Mekhlafi et al., 2021), which was not observed in the juice but was identified as one of the major compounds in fermented beverages.

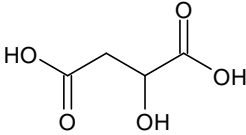
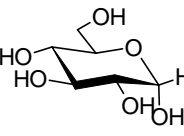
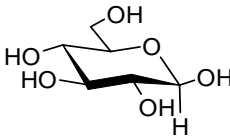
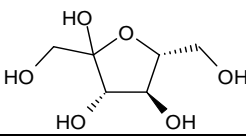
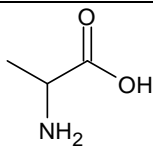
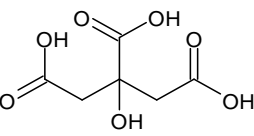
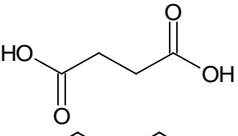
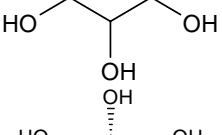
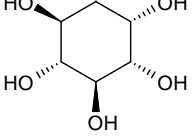
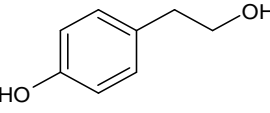
Tyrosol is produced by *Saccharomyces cerevisiae* from L-tyrosine, anaerobically in the essential presence of glucose (Sentheshanmuganathan & Elsdén, 1958). Beer and wine are beverages containing tyrosol. When these beverages are consumed, this simple phenolic compound is converted endogenously to hydroxytyrosol. This phenolic compound has been considered a powerful antioxidant in diets (Soldevila-Domenech et al., 2019). Temperature, alcoholic degree, sugar and amino acid concentrations, oxygen dissolved and pH have been influenced the glycerol biosynthesis, as well as in the production of aromatic compounds (Bordiga et al., 2016; Zhao; Procopio; Becker, 2015).

Figure 3. 1H NMR spectra of *Hylocereus lemairei* fermented beverages extracts (D_2O , 11.74 T).



Source: Authors.

Table 2. Compounds identified in the *Hylocereus lemairei* juice and fermented beverages extracts.

Compounds Structure	Name	NMR Data	
		δ_H in ppm (multiplicity and coupling in Hz)*	δ_C in ppm
Juice			
	malic acid	2.51 (dd, $J = 15.7$ and 8.8), 2.75 (dd, $J = 15.7$ and 3.7), 4.34 (dd, $J = 8.8$ and 3.7)	44.1, 72.4, 181.0, 182.9
	α -glucose	5.23 (d, $J = 3.8$), 3.89 (m), 3.77 (m), 3.45 (m), 3.71 (m), 3.83 (m)	95.1, 72.6, 75.7, 72.7, 63.8, 74.3
	β -glucose	4.64 (d, $J = 8.0$), 3.24 (dd, 9.1 and 8.0), 3.74 (m), 3.47 (m), 3.40 (m), 3.90 (m)	98.9, 77.2, 63.6, 78.7, 72.6, 63.7
	fructose	4.11 (d, $J = 3.7$), 3.82 (m), 3.80 (m), 3.67 (m), 3.57 (m)	77.8, 83.5, 65.3, 65.7, 65.7
Beverages extracts			
	L-alanine	1.36 (d, $J = 7.0$), 4.24 (q)	22.6, 70.5, 183.6
	citric acid	2.88 (d, $J = 15.6$) 2.76 (d, $J = 15.6$)	46.7, 77.0, 177.9, 181.8
	succinic acid	2.62 (s)	32.5, 180.9
	glycerol	3.54 (dd, 11.7 and 6.5), 3.64 (dd, 11.7 and 4.3), 3.77 (m)	65.5, 75.0
	myo-inositol	3.26 (t, $J = 9.5$), 3.52 (dd, $J = 9.5$ and 2.9), 3.61 (t, $J = 9.5$), 4.05 (t, $J = 2.9$).	77.3, 74.1, 75.3, 75.1
	tyrosol	6.86 (d, $J = 8.5$), 7.18 (d, $J = 8.5$)	133.4, 118.4

* Multiplicity: s, singlet; d, duplet; m, multiplet; dd, double duplet; q = quadruplet; triplet. Source: Authors.

3.3 Antioxidant capacity and quantification of total phenols

The results from the antioxidant capacity based on the DPPH and ABTS assays, as well as the quantification of the total phenols are shown in the Table 3. A significant increase of the amount of total phenols was observed in the fermented beverages when compared to the control juice used for their production. Pearson's correlations of DPPH \cdot and ABTS $^{\cdot+}$ radical

scavenging capacity assays were 0.900 (p -value < 0.05). The DPPH and ABTS assays showed excellent Pearson correlations with the total phenols assay: 0.985 and 0.952 (p -value < 0.05), respectively.

The scavenging capacity of the radicals observed from the DPPH and ABTS assays, as well as the presence of phenolic compounds in the fermented beverages can be attributed to the presence of the phenolic compound tyrosol (Souza et al., 2020). The content of phenolic compounds found in the fermented beverages was higher when compared to the fermented beverage with dragon fruit of 28.0 mg GAE L⁻¹ elaborated by Souza and contributors (2018), and lower when compared to the fermented beverage from dragon fruit (*H. costaricensis*) of 280.6 mg GAE L⁻¹ (Jiang et al., 2020).

Table 3. Results of DPPH, ABTS and TPC assays from *Hylocereus lemairei* beverages.

Samples	DPPH (μ M TE)	ABTS (μ M TE)	TPC (mg GAE L ⁻¹)
juice	150 \pm 5 ^c	167 \pm 10 ^b	56 \pm 2 ^c
BB beverage	248 \pm 3 ^a	269 \pm 22 ^a	118 \pm 4 ^a
AC beverage	220 \pm 7 ^b	280 \pm 8 ^a	108 \pm 3 ^b

Results are expressed as means \pm standard deviation (n=3). a-c Different letters in same column are significant (p -value < 0.05). μ M TE = micromolar of Trolox equivalent, mg L⁻¹ GAE = milligram of Gallic acid equivalent per liter of sample. Source: Authors.

4. Conclusion

The physical-chemical parameters analyzed in the *Hylocereus lemairei* fermented beverages prepared with the yeasts BB and AC are in accordance with the current legislation. The chemical profiles of the beverage extracts prepared with yeasts were similar, presenting as major compounds alcohols and organic acids, and phenolic compounds in lower concentration. Tyrosol, a phenolic compound, is probably responsible for the antioxidant capacity of the fermented beverages, when compared to the control juice. There was a significant increase in the antioxidant activity after the fermentation process. Future research must be carried out to evaluate the influence of different parameters in the preparation of *H. lemairei* fermented beverages. The production of new fermented beverage from fruits encourages the cultivation by communities in the capital and interior of Amazonas State, as well as contributes to the formation of local productive arrangements.

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