








# Development and characterization of cupuaçu melomel (*Theobroma grandiflorum*.)

Bianca Gabrielle Oliveira Perote<sup>1\*</sup> , Icaro Rosas Dirane<sup>1</sup> , Charline Soares dos Santos Rolim<sup>1,2</sup> , Leonardo do Nascimento Rolim<sup>1,3</sup> , Jocélia Pinheiro Santos<sup>1,4</sup> , Sérgio Dantas de Oliveira Júnior<sup>1,5</sup> , Wenderson Gomes dos Santos<sup>1,6</sup> 

1 Engenharia de Alimentos, Universidade Federal do Amazonas – UFAM, Campus Setor Sul, Manaus - AM, 69067- 005, Brazil.

2 Programa de Pós-Graduação em Engenharia de Alimentos, Universidade Estadual do Sudoeste da Bahia, Praça Primavera, 40 -Bairro Primavera, Itapetinga -BA, Brazil, 45.700-000.

3 Programa de Pós-Graduação em Biologia de Fungos pela Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE, Brazil, 50670-901.

4 Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Amazonas - UFAM, Av. Gen. Rodrigo Otávio, 6200, Coroado I, Manaus, AM, Brazil, 69080-900.

5 Programa de Pós-Graduação em Engenharia Química, Instituto Nacional de Pesquisas da Amazônia - INPA, Av. André Araújo, Petrópolis, 2935, Manaus, AM, Brazil, 69057-375.

6 Programa de Pós-Graduação em Engenharia de Recursos Naturais da Amazônia, Universidade Federal do Pará, Rua Vera Paz, s/n (Unidade Tapajós) Bairro Salé, Santarém, Pará, Brazil, 68040-255.

\*Corresponding author. Email address: [bgoperote@gmail.com](mailto:bgoperote@gmail.com)

Received 25 October 2023.

Accepted 28 January 2024.

Published 8 March 2024.

**Abstract** - Mead is an alcoholic drink obtained by fermenting a diluted solution of honey, while melomel is a variant of the drink that includes flavorings. Cupuaçu is a fleshy Amazonian fruit with a unique flavor and bioactive properties. The study aims to develop and characterize cupuaçu honey using two commercial yeast strains, *Saccharomyces cerevisiae* (T-58) and *Saccharomyces bayanus* (Red Star). The mead formulations varied between different concentrations of cupuaçu in the must, followed by evaluations including microbiological analyses, physicochemical properties, bioactive compounds and antioxidant capacity. The samples showed no microbial contamination, indicating that the processing followed good manufacturing practices and complied with current legislation. The yeasts used showed normal must attenuation behavior, resulting in an alcohol content of around 9%. There were variations in the levels of phenolic compounds between the samples, with the most significant value being found in the sample using *Saccharomyces bayanus* (Red Star) with 20% cupuaçu, with a content of 14.40%. Therefore, melomel is a technological product that serves as an alternative for honey producers, opening up possibilities for the production of phenolic compounds.

**Keywords:** Regional Fruits. Product Development. Fermented drinks.

## Desenvolvimento e caracterização do melomel de cupuaçu (*Theobroma grandiflorum*.)

**Resumo** - O hidromel é uma bebida alcoólica obtida por fermentação de uma solução diluída de mel, enquanto o melomel é uma variante da bebida que inclui aromas. O cupuaçu é um fruto carnoso da Amazônia com um sabor único e propriedades bioativas. O estudo tem como objetivo desenvolver e caracterizar o mel de cupuaçu utilizando duas linhagens de leveduras comerciais, *Saccharomyces cerevisiae* (T-58) e *Saccharomyces bayanus* (Red Star). As formulações de hidromel variaram entre diferentes concentrações de cupuaçu no mosto, seguidas de avaliações incluindo análises microbiológicas, propriedades físico-químicas, compostos bioativos e capacidade antioxidante. As amostras não apresentaram contaminação microbiana, indicando que o processamento seguiu as boas práticas de fabricação e atendeu à legislação vigente. As leveduras utilizadas apresentaram um comportamento normal de atenuação do mosto, resultando num teor alcoólico de cerca de 9%. Houve variações nos teores de compostos fenólicos entre as amostras, sendo o valor mais significativo encontrado na amostra utilizando *Saccharomyces bayanus* (Red Star) com 20% de cupuaçu, com teor de 14,40%. Portanto, o melomel apresenta-se como um produto tecnológico, servindo como alternativa para os produtores de mel, abrindo possibilidades para a produção de compostos fenólicos.

**Palavras-chave:** Frutas Regionais. Desenvolvimento de Produtos. Bebidas fermentadas.

## Desarrollo y caracterización del cupuaçu melomel (*Theobroma grandiflorum*.)

**Resumen** - El hidromiel es una bebida alcohólica obtenida por fermentación de una solución diluida de miel, mientras que el melomel es una variante de la bebida que incluye aromas. El cupuaçu es un fruto carnoso amazónico con un sabor único y propiedades bioactivas. El estudio pretende desarrollar y caracterizar la miel de cupuaçu utilizando dos cepas comerciales de levadura, *Saccharomyces cerevisiae* (T-58) y *Saccharomyces bayanus* (Red Star). Las formulaciones de hidromiel variaron entre diferentes concentraciones de cupuaçu en el mosto, seguidas de evaluaciones que incluyeron análisis microbiológicos, propiedades físico-químicas, compuestos bioactivos y capacidad antioxidante. Las muestras no mostraron contaminación microbiana, lo que indica que la laboració siguió las buenas prácticas de fabricación y cumplió la legislación vigente. Las levaduras utilizadas mostraron un comportamiento normal de atenuación del mosto, lo que dio lugar a una graduación alcohólica en torno al 9%. Hubo variaciones en los niveles de compuestos fenólicos entre las muestras, encontrándose el valor más significativo en la muestra que utilizó *Saccharomyces bayanus* (Red Star) con 20% de cupuaçu, con un contenido de 14,40%. Por lo tanto, el melomel es un producto tecnológico que sirve como alternativa para los productores de miel, abriendo posibilidades para la producción de compuestos fenólicos.

**Palabras clave:** Frutas Regionales. Desarrollo de Productos. Bebidas fermentadas.

## Introduction

The current situation of beekeepers in communities due to the large extent of the territory of the state of Amazonas, reflects the importance of seeking viable production alternatives that can add value to their products, contributing to income generation and the quality of life of producers.

Brazil is one of the countries with the highest fruit production in the world, according to (FAO 2020), about 14% of food is lost between harvest and sale – in the case of fruits and vegetables, more than 20% is lost, so the post-harvest waste of some crops generates many losses. Thus, there is a need to develop technological processes that allow the reduction of post-harvest losses and at the same time provide an increase in the income of rural producers (Dias 2003; Gomes 2007).

Honey is a natural product, resulting from the processing of flower nectar and other non-flowers parts by bees. This product is widely consumed due to its pleasant taste and because it represents an important source of energy (Uchoa 2016). It can undergo changes in its physical-chemical properties according to the harvest period, possibly attributed to climatic conditions (Okaneku 2020).

Adding value to honey by transforming it into mead can be an alternative for the development of the region. This alcoholic beverage is appreciated in almost everyone with several reported ethnological announced, however, scientific research on it is still uncommon (Teramoto 2005).

However, taking advantage of the biodiversity that the Amazon provides, it is necessary to add flavors and aromas to this drink. Thus, the addition of fruit pulp or juice, such as cupuaçu, provides the development of a unique drink called melomel. The addition of fruits to mead must is a parameter still with little scientific proof in Brazil. Second (Ribeiro 2017), the influence of the addition of fruit to the mead mash is a parameter 16 still little studied in Brazil, like cupuaçu. It is a handcrafted and small-scale beverage, most often by beekeepers. This beverage still does not motivate the commercial interest on the part of the Brazilian beverage industry, whether large, medium or small. In addition to this basic formulation, the must, as this mixture is called, can be added with herbs and/or fruits, generating fermented beverages of the most varied colors and flavors (Vargas 1999; Mcconnell and Schramm 2003).

One of the fruits that can bring pleasant flavors to this drink is cupuaçu, one of the most important typically Amazonian fruits. Its economic value is found in the pulp, which is consumed in the form of juice, nectar, yogurt, ice cream, cream, liquor, pie, jelly, jam, biscuit, ice cream, and other sweets, which, for the most part, are processed by handcraft form, on small production scales. (Cohen e Jackix 2005). According to (Melo *et al.* 2021), due to its characteristics of a great source of vitamin C, which gives it acidic characteristics, and strong anti-inflammatory action, cupuaçu brings many benefits to human health and is of great importance in the diet.

Cupuaçu is also an important food supplement that, lately, has been widely consumed mainly due to its various beneficial properties to health, such as antimicrobial activity, healing properties and antioxidants. (Socha and Pinheiro 2022) says that the fruit has aroused the interest of researchers due to its high economic potential and nutritional values. They are used in the preparation of various food products, as long as the appropriate technology is applied.

Thus, the present work aims to develop and characterize cupuaçu melomel obtained from two strains, *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, proposing a new product for commercialization.

## Material and methods

### Materials

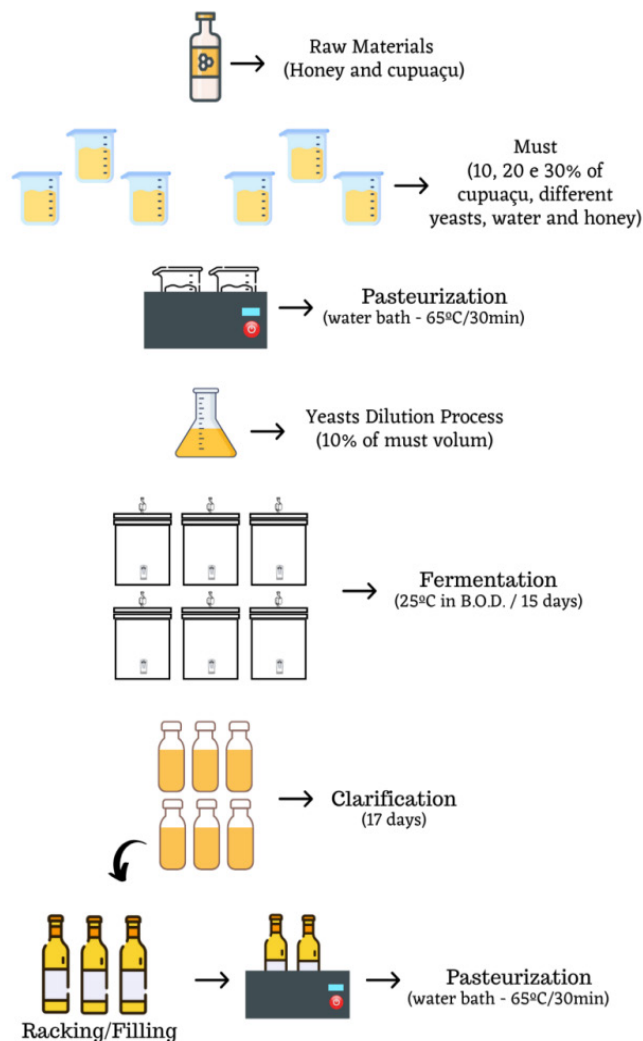
Honey was obtained from Feira do Mel – Flor do Amazonas, while cupuaçu pulp was purchased from local businesses in the city of Manaus - AM, Brazil.

The present study analyzed the fermentative development of the *Saccharomyces cerevisiae* and *Saccharomyces bayanus* strains, the two commercial yeast brands (Fermentis (T-58) and Champgne Red Star (Premier Blanc), respectively, acquired through the Mercado Livre website.

### Products development

The production and characterization of melomel followed the methodology described by (Mattietto *et al.* 2006; Arruda *et al.* 2007), with adaptations. The project was developed in the laboratory of Applied Thermodynamics of the Faculty of Agricultural Sciences of the Universidade Federal do Amazonas, where the fermentative tests were carried out, as shown in Figure 1.

**Figure 1.** Flowchart of melomel processing.



**Source:** Adapted from Arruda *et al.* (2007) and Santos and Santos (2023).

## Hygienization of materials

Following the project planning, all containers and materials were sanitized, washed with water and detergent, in addition to the presence of alcohol at all stages. During the handling of objects, care must be taken not to contaminate them, so the need for a coat, cap, mask, and good laboratory practices.

## Must

A total of 7,500ml of must were produced, divided into 2 batches. In each batch, the honey was diluted in water until it reached a total soluble solids value of 25° Brix, soon after dilution, the must was pasteurized at 65° C for 30 min, table 1 shows the formulations for each fermentation.

**Table 1.** Formulation used to produce the cupuaçu melomel.

Melomel	Formulations		
	Cupuaçu	Must (V/V)	Yeast (P/V)
1-B-1	10%	2250ml	1.125g
1-B-2	20%	2000ml	1g
1-B-3	25%	1875ml	0.9375g
2-B-1	10%	2250ml	1.125g
2-B-2	20%	2000ml	1g
2-B-3	25%	1875ml	0.9375g

The first batch was used with the yeast *Saccharomyces cerevisiae* and the second batch with the yeast *Saccharomyces bayanus*, following the label guidelines, 11.5 g to produce 20 L.

## Yeast dilution

In order to increase the number of yeasts and accelerate the fermentation process, 3 yeasts dilution process were produced for each batch, using *Saccharomyces cerevisiae* yeasts for the first batch (brand T-58) and *Saccharomyces bayanus* (brand Red Star). The first batch was used with *Saccharomyces cerevisiae* yeast and the second batch with *Saccharomyces bayanus* yeasts, following the label guideline, 11,5 g to produce 20 L.

## Clarification

Following the label guidelines (25 ml to each 2g), the amounts of gelatin for each melomel were calculated, as shown in Table 2. At this moment, the melomel was transferred to the containers where the clarification occurred, during the following days the homogenization of the melomel was observed.

**Table 2.** Amounts of clarifier for each melomel sample.

Melomel	1	2	3
CLARIFYING	12 ml	10.2 ml	8.4 ml

### Filling and pasteurization

After 17 days of clarification, the material from the first batch was transferred to sanitized, sealed and pasteurized glass bottles again at 65°C for 30 min.

### Microbiological assessment

The analyzes were made following the methodologies of (MAPA 2003). When microbiologically analyzing the samples, 3 culture media were required: one simple for microorganisms in general, one selective for gram-negative bacteria (coliforms) and one selective for fungi. The media used were Nutrient Agar (simple, for general growth), MacConkey Agar (selective, for gram-negative bacteria, observation of coliforms) and Nutrient Agar plus 0.1% chloramphenicol (selective, for fungal analysis).

Samples were contained in 50 ml falcon tubes and stirred for homogenization. 100µl of each sample were inoculated into petri dishes with their respective culture media. With the aid of the drigalski spatula, the inoculum was spread over the entire surface of the dish.

The analysis was performed in duplicates, after the inoculations of the dishes, they were taken to a bacteriological oven at 37°C for 48 hours. After the bacteriological oven period, the samples are taken to verify the microbiological development.

### Physicochemical analysis

After the two batches produced and fermented, the physicochemical characteristics were analyzed, according to the official methodologies of the Adolfo Lutz Institute (IAL 2008). All analyses were expressed as mean ± standard deviation of percent inhibition.

**Total titratable acidity:** Total acidity quantifies how acidic or sour the food tastes. Analysis carried out according to A.O.A.C. methodologies (1992):

**Volatile Acidity:** the sample is titrated before (total acidity) and after evaporation (non-volatile or fixed acidity), and by difference between the titrations we have the % of volatile acidity (EMBRAPA 2010):

$$\text{Volatile acidity} = \text{total acidity} - \text{fixed acidity}$$

**Fixed Acidity** the determination of fixed acidity, through the calculation taken from (Felisbino 2017):

$$AC = TA - VA$$

TA= total acidity

VA= Volatile acidity

**pH** with the pHmeter aid;

**Total Soluble Solids (TSS)** with refractometer aid;

**Alcohol content:** The determination of the % of alcohol by volume was carried out according to (Lima 2021).

**Ash:** Corresponds to the residue from the incineration of the sample, taken from (Felisbino 2017), carried out using 20 ml of each formulation and taken to the muffle, in triplicate, at 550°C.

#### **Analysis of *in vitro* antioxidant activity**

To analyze the antioxidant activities, the extracts were obtained, where the material was initially subjected to lyophilization to proceed with the evaluation of phenolic compounds and antioxidant activity. After lyophilization, samples were suspended from DMSO (10 mg/ 1 mL).

#### **Quantification of Total Phenolic Compounds (TPC)**

The content of total phenolic compounds was estimated according to (Pires 2017), using the Folin-Ciocalteu reagent. To this, 20 µL extracts, 200 µL of water, 20 µL of Folin-Ciocalteu reagent and 60 µL of 10% sodium carbonate solution were added. After homogenization, the reaction mixture remained under rest for 30 min, protected from light and, subsequently, spectrophotometric readings (SYNERGY H1, BioTek, USA) were taken at 760 nm. Gallic acid was used as standard and the results expressed as micrograms of Gallic Acid Equivalence (GAE) per mg of sample.

#### **Quantification of flavonoids**

The flavonoid content was evaluated according to the methodology described by (Chang 2012), where the sample (25 µL) was homogenized together with water (152.5 µL) and sodium nitrite (5%, 7.5 µL). After 6 minutes, aluminum chloride (10%, 15 µL) was added and incubated for 5 minutes at room temperature. Finally, sodium hydroxide (1 M, 50 µL) was added and the reaction mixture was incubated for 15 minutes at room temperature. With the aid of a microplate reader, the absorbance was recorded at 510 nm, and the results were expressed in micrograms of equivalence with quercetin (EQ) per mg of sample.



## Antioxidant potential of extracts

The antioxidant activity of the extracts was determined according to the methods of DPPH•, ABTS•+, reducing power and chelating ability, according to methodologies adapted for 96-well microplates, by (Khatua *et al.* 2017).

### DPPH radical inhibition

In this assay, 180  $\mu\text{L}$  of DPPH• (2,2-diphenyl-1-picrylhydrazyl, 4 mg.mL<sup>-1</sup>) was incubated with 20  $\mu\text{L}$  of the extracts for 30 minutes, in the dark. After the incubation time, absorbances were obtained at a wavelength of 595 nm.

### ABTS•+ radical inhibition

For the determination of the antioxidant potential by the method employing ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid), initially, a stock solution of radical ABTS was prepared by the reaction of ABTS (7 mM) and potassium persulfate (2.45 mM) for 16 hours, in the dark, at room temperature. Subsequently, the solution was diluted in ethanol to an absorbance between 0.8 and 1.0 at 754 nm. The assay consisted of incubating 180  $\mu\text{L}$  of ABTS•+ with 20  $\mu\text{L}$  of the extracts for 5 minutes in the dark, with subsequent recording of absorbance at 754 nm. Results were expressed as mean  $\pm$  standard deviation of percent inhibition.

### Chelating Ability

The determination of the chelating ability on the Fe<sup>2+</sup> ion was performed by incubating 100  $\mu\text{L}$  of the samples with 5  $\mu\text{L}$  of iron chloride II (3 mM), 10  $\mu\text{L}$  of ferrozine solution (0.12 mM) and 85  $\mu\text{L}$  of distilled water for 15 minutes, with subsequent spectrophotometric readings at 562 nm. Results were expressed as mean  $\pm$  standard deviation of percent inhibition.

### Reducing Power

For the determination of the reducing power, 10  $\mu\text{L}$  of the samples were incubated with 25  $\mu\text{L}$  of phosphate buffer (0.2 M, pH 6.6) and 25  $\mu\text{L}$  of potassium ferrocyanide (1%), for 20 minutes, at room temperature (25  $\pm$  2 °C). Subsequently, 25  $\mu\text{L}$  of trichloroacetic acid (10% v:v), 85  $\mu\text{L}$  of distilled water and 8.5  $\mu\text{L}$  of iron chloride III were added, performing an incubation for another 15 minutes and consecutive reading at 750 nm. The results were expressed in absorbance units, where a higher value indicates greater reduction power of the samples.

## Results and Discussion

After complete fermentation of the melomel, the formulations were subjected to microbiological, physicochemical and antioxidant analyses. For all the analyzes carried out, the results found respect what the legislation recommends.



## Microbiological evaluation

For the three culture media, the results were negative for contamination, remembering that the Nutrient Agar culture medium presents microorganisms in general, MacConkey Agar shows if there are gram-negative bacteria, specifically *Escherichia coli* (fecal contamination bioindicator) and Nutrient Agar with addition of 1% chloramphenicol, for observation of fungi only.

It was observed that this production presented good results and maintained the characteristics of the product according to the current legislation, for both yeasts. According to (Alves 2009) this absence of the microorganisms studied may have been favored by the pH range found in the samples analyzed by the pH range found in the samples analyzed. It can also be said that these negative results are related to the acidic pH of the drink, as well as the microbiological analyses, which are the result of good laboratory practices in the handling and production of melomel.

Translated with DeepL.com (free version) It can be said that these negative results presented in relation to the microbiological analyses are the result of good laboratory practices for handling and production of melomel.

## Physicochemical Evaluation

Through the physicochemical analyzes performed on melomel, the characteristics shown in Table 3 were analyzed. For the total acidity, volatile acidity and fixed acidity indices, according to Normative Instruction N°. 34 of November 29 (MAPA 2012), satisfactory results were obtained for all samples.

The total acidity results were similar to those found by (Pereira 2014) and (Lima 2021), which produced cupuaçu mead and melomel, respectively. Pereira found acidity of 87.77 meq/L while Lima found total acidity of 50.12 meq/L for T-58 yeast and 62.17 meq/L for Red Star yeast

In the first batch, carried out with the yeast *Fermentis* (T-58), it was observed that there was an increase in total acidity of 21.10 meq/L when the concentration of cupuaçu pulp was increased from 10% to 20%, the total acidity value for the concentration of 25% remained unchanged. Nevertheless, the second batch carried out with the Red Star yeast (*premier blanc*) it was possible to analyze a difference of only 2.3 meq/L when the concentration of cupuaçu was increased from 10% to 20%, the constant remained the same total acidity value for a concentration of 25%, the relatively smaller difference compared to melomel produced with the yeast *Fermentis* (T-58).

**Table 3.** Mean values  $\pm$  standard deviation, analyzing the total acidity, volatile acidity and fixed acidity of melomel samples.

Parameters	AT= Total Acidity	VOLATILE ACIDITY	FIXED ACIDITY
1-B-1	72.7 <sup>b</sup> $\pm$ 0.050	7.7 <sup>a</sup> $\pm$ 0.3536	64.9 <sup>b</sup> $\pm$ 3.6670
1-B-2	93.8 <sup>a</sup> $\pm$ 0.000	5.5 <sup>a</sup> $\pm$ 0.7071	88.3 <sup>a</sup> $\pm$ 0.7071
1-B-3	93.8 <sup>a</sup> $\pm$ 0.000	5.2 <sup>a</sup> $\pm$ 0.3535	88.5 <sup>a</sup> $\pm$ 0.3535
2-B-1	96.1 <sup>a</sup> $\pm$ 0.050	5.0 <sup>b</sup> $\pm$ 0.0000	91.1 <sup>a</sup> $\pm$ 3.3163
2-B-2	93.8 <sup>a</sup> $\pm$ 0.000	5.0 <sup>b</sup> $\pm$ 0.0000	88.8 <sup>a</sup> $\pm$ 0.0000
2-B-3	93.8 <sup>a</sup> $\pm$ 0.000	7.0 <sup>b</sup> $\pm$ 0.7071	86.7 <sup>a</sup> $\pm$ 0.0707
FCal	42.8	22.073	45.96
CV%	8.917	19.89	11.17

The results presented refer to the means of the determinations in triplicates, followed by the respective standard deviation (Analysis of variance - ANOVA and Tukey's test).

There was a difference of 23.4 meq/L between the total acidity result of the first formulation of the 1st batch compared to the first formulation of the 2nd batch. It can be stated that only experiment 1-B-1 (10% cupuaçu pulp and T-58 yeast) differed statistically from the averages of the other experiments, for total and fixed acidity, indicating a product with lower acidity.

Analyzing the pH values in Table 4, the variations between the samples were very small, being less than four and greater than three ( $3.48 < \text{pH} < 3.66$ ). The analysis of variance showed that there is no statistically significant difference between the means for this parameter, that is, even with lower pH, the transformation of sugars into alcohol was stabilized.

It was verified that both the *Saccharomyces cerevisiae Fermentis strain* (T-58) and the Red Star champagne strain (premier blanc) processed the substrate well and transformed it into a product (alcohol) obtaining values approximating 9%, respecting the values found in the legislation, all samples presented values between 4 and 14%, according to IN N<sup>o</sup>. 34 (MAPA 2012).

The total soluble solids values for the two batches showed little differentiation, and can be classified according to Normative Instruction N<sup>o</sup>. 34, of November 29 (MAPA 2012) as mild melomel.

**Table 4.** Mean values  $\pm$  standard deviation of the physicochemical parameters of the cupuaçu melomel samples.

Parameters	Ash (g/L)	TSS (°Brix)	pH (24°C)	Alcohol content (%)
1-B-1	4.12 <sup>a</sup> $\pm$ 0.10	8.5 <sup>c</sup> $\pm$ 0.00	3.57 <sup>a</sup> $\pm$ 0.010	8.92 <sup>c</sup> $\pm$ 0.000
1-B-2	5.04 <sup>a</sup> $\pm$ 0.68	7.75 <sup>b</sup> $\pm$ 0.05	3.48 <sup>a</sup> $\pm$ 0.005	9.32 <sup>b</sup> $\pm$ 0.038
1-B-3	4.59 <sup>a</sup> $\pm$ 0.39	7.0 <sup>a</sup> $\pm$ 0.00	3.54 <sup>a</sup> $\pm$ 0.005	9.73 <sup>a</sup> $\pm$ 0.000
2-B-1	5.12 <sup>a</sup> $\pm$ 0.54	7.0 <sup>a</sup> $\pm$ 0.05	3.52 <sup>a</sup> $\pm$ 0.005	9.70 <sup>a</sup> $\pm$ 0.038
2-B-2	6.12 <sup>a</sup> $\pm$ 0.86	7.8 <sup>b</sup> $\pm$ 0.00	3.66 <sup>a</sup> $\pm$ 0.010	9.30 <sup>b</sup> $\pm$ 0.000
2-B-3	8.92 <sup>a</sup> $\pm$ 1.80	7.0 <sup>a</sup> $\pm$ 0.00	3.64 <sup>a</sup> $\pm$ 0.005	9.73 <sup>a</sup> $\pm$ 0.000
FCal	3.684	424.6	2.85	424.6
CV (%)	32.25	7.40	2.12	40.23

The results presented refer to the means of the determinations in triplicates, followed by the respective standard deviation (Analysis of variance - ANOVA and Tukey's test).

When analyzing the total soluble solids of the meads, the difference ( $p < 0.05$ ) between the two samples (1-B-2 and 2-B-1) was verified, which indicates that there was an efficient conversion of sugars into ethanol by the two strains of *Saccharomyces cerevisiae* during fermentation, since the musts used were at about 24°C. In the first batch, the final TSS values were inversely proportional to the cupuaçu concentrations, the beverage with 10% cupuaçu obtained the highest TSS (8.5 °Brix), different from the sample with 25% cupuaçu, which had the lowest TSS (7 °Brix). Taking into account that the must started with total soluble solids of 25 °Brix, it is stated that the decrease in the solids content presents the conversion of sugar into alcohol during the process, there was a satisfactory consumption of substrate and it is possible to notice the reduction of sugar levels and alcoholic growth.

Ash analysis determines the mineral constituents of food, the study by (Cecchi 2003) states that some metal residues can cause toxic effects such as Pb and Hg, the oxidation of ascorbic acid (Vitamin C) and the instability of fruit juices are affected by Cu. Some mineral components may enhance and others prevent fermentation of fermented products. The results found (4.12 to 8.92 g/L) for ash fit the parameters imposed for melomel, respecting the minimum limit of 1.5 for ash. In general, all the analyses showed satisfactory results that comply with Decree N°. 6,871 of June 4 (Brasil 2009).

### Analysis of *in vitro* antioxidant activity

Table 5 presents the data regarding the antioxidant potential of the mead samples.

**Table 5.** *In vitro* antioxidant activity analyses of melomel with different yeast and cupuaçu formulations.

Parameters	Reducing Power (750nm)	Phenolic Compounds	Flavonoids	DPPH* (%)	ABTS+• (%)
1-B-1	0.078 <sup>a</sup> ±0.004	5.78 <sup>a</sup> ±2.52	1.41 <sup>b</sup> ±0.61	20.95 <sup>c</sup> ±0.38	23.28 <sup>c</sup> ±1.49
1-B-2	0.154 <sup>a</sup> ±0.014	4.82 <sup>a</sup> ±0.68	0.52 <sup>a</sup> ±0.04	17.96 <sup>b</sup> ±1.83	38.82 <sup>a</sup> ±0.50
1-B-3	0.102 <sup>ab</sup> ±0.007	6.38 <sup>a</sup> ±0.58	0.49 <sup>a</sup> ±0.05	16.40 <sup>ab</sup> ±2.11	41.15 <sup>a</sup> ±2.90
2-B-1	0.112 <sup>ab</sup> ±0.014	5.82 <sup>a</sup> ±0.39	-0.30 <sup>a</sup> ±0.03	17.73 <sup>ab</sup> ±1.34	38.33 <sup>a</sup> ±0.11
2-B-2	0.079 <sup>b</sup> ±0.011	14.40 <sup>b</sup> ±3.53	0.48 a±0.03	-49.08 <sup>a</sup> ±2.47	43.04 <sup>ab</sup> ±3.20
2-B-3	0.094 <sup>c</sup> ±0.013	12.12 <sup>b</sup> ±1.48	0.58 <sup>c</sup> ±0.13	11.36 <sup>a</sup> ±3.49	49.22 <sup>b</sup> ±3.31
FCal	18.62	16,99	13.43	407.85	43,45
CV (%)	10.18	18.59	22.47	37.00	4,92

The results presented refer to the means of the determinations in triplicates, followed by the respective standard deviation (Analysis of variance - ANOVA and Tukey's test).

Secondary metabolites are not necessarily produced in all conditions and, in most cases, the functions of these substances in the body are not yet known. However, due to their immobility, plants have developed means to defend themselves against some herbivores, compete with other plants, against pathogens, to deal with climate change, sunlight intensity and nutrient deficiency. They also produce metabolites to attract pollinating insects and seed dispersers, playing some vital role for the well-being of the producer (Dewick 2002).

In the analyses of reducing power, it was observed that the parameter 1-A-2 (0.154) presented the highest potential, followed by 2-B-1 (0.112) and 1-A-3 (0.102). Parameters 2-B-2 and 2-B-3 presented the highest contents of phenolic compounds in their compositions, with 14.40% and 12.12%, respectively. In the quantification of flavonoids in each sample, the highest contents were observed in 1-A-1 (1.41%), followed by 2-B-3 (0.58%) and 1-A-2 (0.52%). In the reactions against DPPH• and ABTS+ • radicals, none of the samples showed to be able to inhibit at least 50% of the radicals. However, with the exception of parameter 2-B-2, which presented a negative value, all were able to react with the free substances in the solution. Sample 2-B-3 inhibited 49.22% of ABTS+ • radicals, being the most promising parameter among the others.

The antioxidant activity of phenolic compounds is justified by the reduction-oxidation properties, which allow them to reduce singlet oxygen species (O•), by releasing protons (H<sup>+</sup>) in the reaction medium, maintaining their stabilization by resonance, presenting the hydroxyls in the *para* (C-4) position as the most active. Flavonoids, on the other hand, depend on the amount of hydroxyls present in the structure, other substitutions and conjugations. (di Majo *et al.* 2005; Caiet *al.*2006; Coutinhoet *al.* 2008).

Although the analyzes show the presence of phenolic compounds in all samples, they did not show the ability to inhibit 50% or more of the radicals present in the medium. This fact can be justified by the nature of phenolics, which may lack free acidic hydroxyls to release protons (H<sup>+</sup>), as is the case with glycosylated flavonoids.

## Conclusion

It can be concluded that the cupuaçu honey produced with *Saccharomyces cerevisiae* and *Saccharomyces bayanus* showed physicochemical and microbiological characteristics within the standards of current legislation, presenting itself as a technological product, serving as an alternative for honey producers in the market, opening up possibilities for the production of phenolic compounds. In addition, it can be inferred that the *Saccharomyces cerevisiae* yeast (Red Star brand) was able to produce a drink with a higher acidity content. For the microbiological analyses, the results were free of contamination, showing that the processing was carried out in accordance with good food formulation practices. There were variations in the content of phenolic compounds between the samples, with the most significant value being found in the sample that used *Saccharomyces bayanus* (Red Star brand) with 20% cupuaçu, with a content of 14.40%.

**Author participation:** BGOP - Conceptualization, data curation, formal analyses, funding acquisition, investigations, methodology, resources, software, supervision, validation, writing - original draft, writing - review & editing; IRD - Conceptualization, investigations, resources; CSSR, LNR, JPC, SDOJ - Investigations, methodology, resources; WGS - Supervision, resources, Writing - Conceptualization, data curation, formal analyses, methodology, resources, validation, software, writing - original draft, writing - review & editing;

**Ethical approval:** Ethical approval was not required for this study. The entire process was carried out at the Applied Thermodynamics Laboratory, which is coordinated by Professor Wenderson Gomes dos Santos, the supervisor of this article. There was no need for external approval.

**Funding:** BGOP was a PIBIC-CNPq scholarship holder (2021 - 0223). Laboratory of Applied Thermodynamics FCA/UFAM for its support in the development of the project.

**Data availability:** no data is deposited in any database or repositories.

**Conflict of Interest:** No conflict of interest.

## References

- Instituto Adolfo Lutz. 2008. Métodos físico-químicos para análise de alimentos. 4 ed. (1ª digital edição), 1020p.
- Alves EM, Toledo VAA, Marchini LC, Sereia MJ, Moreti ACCC, Lorenzetti ER, Neves CA, Santos AA. 2009. Presence of coliforms, molds and yeasts in samples of organic honey from Africanized bees from the islands of the upper Paraná River. Rural Science, Santa Maria, 39(7): 2222- 2224. DOI: <https://doi.org/10.1590/S0103-84782009005000140>.
- Arruda AR, Casimiro ARS, Garruti DS, Abreu FAP. 2007. Physicochemical characterization and sensory evaluation of fermented alcoholic banana beverage. Revista Ciências Agronômicas 38(4): 377- 384.
- AOAC. 1992. Official methods of analysis of the Association of Official Analytical Chemistry International. 13ed, Washington,
- Brasil. 2009. Decreto Nº 6.871, de 4 de junho de 2009. Dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas.
- Brunello LT, Imaizumi VM, Venturi Filho WG. 2017. Physicochemical, energetic and sensory characterization of mead. Energy in Agriculture 32(2): 200-208. DOI: <https://doi.org/10.17224/EnergAgric.2017v32n2p200-208>
- Cecchi, HM. 2003. Fundamentos teóricos e práticos da análise dos alimentos. 2ª ed. Campinas - SP. Editora Unicamp, 206p.

- Chang CL, Lin CS, Lai GH. 2011. Phytochemical characteristics, free radical scavenging activities, and neuroprotection of five medicinal plant extracts. *Evidence-Based Complementary and Alternative Medicine*, 2012(1): 8p. DOI: <https://doi.org/10.1155/2012/984295>.
- Cohen, KO, Jackix MNH. 2005. Estudo do Liquor de Cupuaçu. *Food Science and Technology*. 25(1): 182-190. DOI: <https://doi.org/10.1590/S0101-20612005000100030>.
- Cai YZ, Sun M, Xing J, Q L, Corke H. 2006. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences* 78(25): 2872-2888. DOI: 10.1016/j.lfs.2005.11.004.
- Coutinho ID, Coelho RG, Kataoka VMF, Honda, NK, Silva JRM, Vilegas W, Cardoso CAL. 2008. Determination of phenolic compounds and evaluation of antioxidant capacity of *Campomanesia adamantium* leaves. *Ecletica Quimica*, 33(4): 53-60. DOI: <https://doi.org/10.1590/S0100-46702008000400007>.
- Di Majo D, Giammanco M, Guardia LA, Tripoli E, Giammanco S, Finotii E. 2005. Flavanones in Citrus fruit: Structure-antioxidant activity relationships. *Food Research International*, 38(10): 1161-1166. DOI: <https://doi.org/10.1016/j.foodres.2005.05.001>.
- Felisbino LM, Campos DP, Marques CRM. 2017. Caracterização físico-química da fermentação alcoólica de diferentes cortes de uva na produção de vinhos artesanais. *Revista Vincici* 2(2): 25-51.
- Khatua S, Ghosh S, Acharya K. 2017. A simplified method for microtiter based analysis of *in vitro* antioxidant activity. *Asian Journal of Pharmaceutics* 11(20):327-335.
- Lima, LLB. 2019. Perfil socioeconômico e conhecimento dos parâmetros de qualidade por parte dos produtores e consumidores de mel no Distrito Federal. Monografia (Graduação em Agronomia). UNB - Brasília, 52p.
- Lima LFFS, Alves TCL, Souza AQL, Santos WG. 2021. Kinetic analysis of the fermentative process of mead with the addition of cupuaçu (*Theobroma grandiflorum*) pulp. *Research, Society and Development* 10(14). DOI: <http://dx.doi.org/10.33448/rsd-v10i14.21685>.
- Lima MCF. 2021. Caracterização de substâncias fenólicas e alcalóides de resíduos de cupuaçu (*Theobroma grandiflorum* (Willd. ex Spreng.) Schum). Dissertação de Mestrado, UFAM – Amazonas.
- Mattietto RA, Lima FCC, Venturieri GC, Araújo AA. 2006. Tecnologia para Produção Artesanal de Hidromel Doce. Comunicado Técnico (170) – EMBRAPA.
- McConnell DS, Schramm KD. 1995. Mead success: ingredients, processes and techniques. Zymurg Spring, v. 4.
- Melo FS, Okaneku BM, Cardoso DP, Rodrigues EC, Santos WG. 2021. Avaliação das Características físico-químicas de polpa e concentrado de cupuaçu (*Theobroma grandiflorumschum*) da região Amazônica. *Brazilian Journal of Development*. 7(1): 10462-10472. DOI:10.34117/bjdv7n1-712.
- EMBRAPA. Metodologia para Análise de Vinho, 2010 / editor técnico: Rizzon LA – Brasília, DF: Informação Tecnológica , 120p.
- Ministério da Agricultura, Pecuária e Abastecimento (MAPA). 2003. Instrução Normativa N° 62, de 26 de agosto de 2003. Métodos Analíticos Oficiais para Análise Microbiológica para Controle de Produtos de Origem Animal e Água.
- Ministério da Agricultura, Pecuária e Abastecimento (MAPA). 2012. Instrução Normativa N° 34, de 29 de novembro de 2012. Estabelecer padrões complementares de identidade e qualidade para bebidas fermentadas.
- Okaneku BM, Souza AQL, Araújo DL, Alves TC, Cardoso DNP, Santos WG. 2020. Análise Físico-química e microbiológica do mel de abelhas africanizadas (*Apis mellifera*). *Brazilian Journal of Development*. 6(4): 18607-18620. DOI: <https://doi.org/10.34117/bjdv6n4-144>.
- Food And Agriculture Organization of the United Nations (FAO). 2021. FAO and PNUMA call for a movement in Brazil to reduce food losses and waste.

Pereira AS, Costa RAS, Landim LB, Silva NMC, Reis MFT. 2014. Produção de Fermentado Alcolóico Misto de Polpa de Açaí e Cupuaçu: Aspectos Cinéticos, físico-químicos e Sensoriais. Revista Brasileira De Tecnologia Agroindustrial 8(1):1216-1226. D.O.I.: 10.3895/S1981-36862014000100007.

Pires JS, Torres PB, Santos DYAC, Chow F. 2017. Ensaio em Microplaca de Substâncias Redutoras pelo Método do Folin-Ciocalteu para Extratos de Algas. Instituto de Biociências, Universidade de São Paulo. DOI: 10.13140/RG.2.2.29127.80809.

Ribeiro LHL. 2017. Produção e Caracterização de Hidromel de Frutas Vermelhas. Monografia (Engenharia de Alimentos). UFMA - Maranhão, 36p.

Santos SS, Santos WG. 2023. Fabricação de Melomel Saborizado com Taperebá (*Spondias mombin* L. Revista Perspectiva, 47(179): 83-92. <https://doi.org/10.31512/persp.v47.n.179.2023.348.p.83-92>

Socha LB, Pineiro RBM. 2016. Cupuaçu: a fruta globalizada. Revista Gestão e Sustentabilidade Ambiental 4(2): 554-567. DOI: <https://doi.org/10.19177/rgsa.v4e22015554-567>.

Teramoto Y, Sato R, Ueda S. 2005. Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. African Journal of Biotechnology 4(2):160-163.

Uchoa MLP, Gonsalves HO, Silva KFN, Souza GC. 2016. Avaliação Microbiológica de Mel de Abelha (*Apis mellifera*) Comercializados em Supermercados da Cidade de Limoeiro do Norte -CE. VI Encontro Internacional de Jovens Pesquisadores. IFCE- campus Limoeiro do Norte.

Vargas P, Gulling R. 1999. Making Wild Wines and Meads: 125 unusual recipes using herbs, fruits, flowers and more. United States: Storey Publishing.

Wiese H. 2005. Apicultura: novos tempos. 2. ed. Guaíba: Ed. Agropecuária, 378p.



Esta obra está licenciada com uma *Licença Creative Commons Atribuição Não-Comercial 4.0 Internacional*.