



Evaluation of the Physicochemical and Microbiological Stability of Cocoa Honey-Flavored Mate Tea under Different Storage Conditions

Kamila Alves Santos^{1*}, Josué Castro de Jesus², Valéria Ruama Trindade Martins³, Adrielle Oliveira da Cruz³, Rita de Cássia de Souza Costa³, Thâmilla Thalline Batista De Oliveira³

¹ Federal University of Bahia, Bachelor of Health Science, Salvador, Bahia, Brazil

² COOPERMATA (Cooperativa Cacau Mata Atlântica da Bahia), Ituberá, Bahia, Brazil

³ SENAI CIMATEC; Industrial Microbiology, Salvador, Bahia, Brazil

*Corresponding author: Federal University of Bahia; addresses; kamila.santos@ufba.br

Abstract: Cocoa is a versatile fruit, used by the food industry mainly for the production of chocolate, and cocoa honey, liquid extracted during the fermentation or pressing of cocoa, when combined with mate tea, results in a drink with remarkable sensory and commercial appeal. This study aimed to evaluate the physicochemical, microbiological and sensory stability of cocoa honey mate tea stored under two temperature conditions: ambient (CM1) and refrigerated (CM2), over 45 days. The results showed that the microbiological indices remained within acceptable limits throughout the storage period, ensuring food safety. The physicochemical analysis showed stability of titratable acidity in CM2, while CM1 presented slight reduction. The pH remained stable in both samples, similar to previous studies with mate tea-based beverages. There was a reduction in °Brix values in both conditions. The vitamin C content decreased in CM2 and increased in CM1. Sensory analysis indicated high acceptance in all attributes, without significant differences between the samples over time. In the face of this, it is important to continue the study to assess whether stability is maintained.

Keywords: Stability. Cocoa honey. Mate tea.

1.Introduction

Cocoa (*Theobroma cacao* L.) is a tropical fruit of high economic and cultural value, with wide use in the food industry, cosmetics and pharmaceuticals (Guirlanda, 2021 [9]). According to the International Cocoa Organization (ICCO, 2025 [12]), Brazil stands out among the seven countries that produce most cocoa, with Bahia and Pará being the states with the highest production quantities.

Among the by-products of the fruit is cocoa honey, a liqueur extracted by pressing or during fermentation. Just like cocoa, cocoa honey can be used in different ways, for example as a base for jellies, fermented drinks, sweetener or juices. One of the ways to use it is combined with mate tea, which, according to Portaria N° 519 of June

26, 1998, are products used in the preparation of food beverages, resulting from different technological processes and consisting of parts of vegetables, whole, fragmented or ground (Brazil, 1998 [4]).

It should also be mentioned that, according to the 2017-2018 IBGE Family Budget Survey (POF), only 7.4% of the Brazilian population consumes tea daily (IBGE, 2020 [11]). The association between mate tea and cocoa honey represents an innovative alternative for increasing consumption and adding value to regional ingredients, after all, the combination results in a refreshing drink with sensory and commercial acceptance.



However, its greatest challenge is the short shelf life, due to the high microbial load present in the fruit and the post-harvest conditions. These factors make cocoa honey and, consequently, beverages that have it in their formulation, highly perishable (Guirlanda, 2021 [9]).

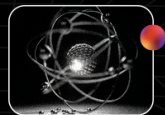
Being a promising product, the physical-chemical, microbiological and sensory study of cocoa honey allows to develop methods that extend its shelf life and expand industrial applications. Analyzing samples periodically and under different conditions contributes both to the diversification of the market, as for quality assurance and product safety (Grizotto, 2006 [10]).

This study goes beyond the proposal of using cocoa honey, by directing to the development of a functional drink from this by-product, in order to expand consumption alternatives, promote the appreciation of regional biodiversity and add value to an ingredient still underused in the food industry. In this sense, it is essential to evaluate the influence of different storage conditions, at room temperature (CM1) and refrigerated (CM2), on the physico-chemical, microbiological and sensory stability of the formulation over time. Thus, it becomes necessary to evaluate the effects of different storage temperatures, tea mate cocoa honey flavor CM1 and CM2 over a period.

This study aimed to evaluate the stability of cocoa honey mate tea, stored at room temperature (CM1) and refrigerated (CM2), over 45 days, through physical-chemical, microbiological and sensory analysis.

2. Methodology

To determine the stability of cocoa honey mate tea (CM1 and CM2), this study evaluated two samples of the product over 45 days under different storage conditions. The samples were kept at room temperature and refrigerated, being analyzed over 45 days. The microbiological characterization evaluated the presence or absence of fungi, molds and yeasts, according to the limits established by the Normative Instruction no 161/2021 of Brazilian Health Regulatory Agency (ANVISA) [3], ensuring that the product complies with the quality and safety standards required for ready-to-drink beverages. The physicochemical analyses were pH, using digital pHmeter (PHS3BW, Bel Engineering), portable digital refractometer (SPINLAB/SPIN104-D) for measurement of °Brix and official methods for determination of vitamin C (EN 014 FQA - MAPA IN 24, 2005 [5]) and titratable acidity (EN 019 FQA - IAL 312/IV [1]). These tests allowed to evaluate the influence of physical-chemical parameters on the stability and shelf life of the product, ensuring quality, standardization and compliance with regulatory requirements. Finally, the



sensory analysis was carried out according to ABNT NBR ISO 11136:2022 [6], by means of hedonic tests in a controlled environment, to verify the sensory acceptance and the purchase intention, comparing the newly produced with those stored under different conditions during the study period.

3. Results and Discussion

The results of the microbiological analyses (Table 1) performed in CM1 and CM2 tests were considered satisfactory, since they are within the limits established by RDC no 724 and by Normative Instruction no 161, both dated July 1, 2022 (Brazil, 2022 [2]).

Table 1. Result of microbiological analysis in the different storage times of tea mate cocoa honey flavor at room temperature and chilled.

Microrganism	Sample	T0	T45
Fungus, Molds, and Yeast (mL/g)	CM1	<1,0 X 10 est.	<1,0 X 10 est.
	CM2	<1,0 X 10 est.	<1,0 X 10 est.

Food contamination can occur through the environment, water, utensils, containers, raw materials and carelessness of handlers, and it impacts the shelf life of foods (Pereira and Braga, 2014 [15]). Therefore, to evaluate this shelf life, microbiological analyses are essential. Due to the presence of cocoa honey as one of the ingredients, which has a high microbial load

(Guirlanda, Silva and Takahashi, 2021 [9]), product perishability was taken into account. In view of this, the previous heat treatment of cocoa honey allowed the preservation of its properties. In addition, the incorporation of additives and preservatives in the formulation of cocoa honey mate tea did not negatively affect the sensory acceptance of the product by consumers, on the other hand, contributed to a significant increase in shelf life, extending the period in which the product can be marketed without loss of quality.

The levels of fungi, molds and yeasts remained within the limits allowed by IN N° 161 (Brazil, 2021 [3]) at all different times. This compliance with microbiological standards guarantees the safety of the food for the consumer and product quality recognized in the market. Thus, good manufacturing practices, together with the use of additives and preservatives, played a crucial role in the results of cocoa honey mate tea, even after 45 days stored at room temperature or refrigerated.

Table 2 presents the physico-chemical results of CM1 and CM2 samples, observed during this period. It is observed that the titratable acidity presented similar values in T0 and T45 at refrigerated temperature, while the ambient temperature decreased, varying 0.66%. At ambient temperature, the observed variability may have occurred due to the chemical alteration of the CM2, caused by the action of



microorganisms that degrade the organic acids present, reducing the acidity over time. On the other hand, due to the more effective storage conditions in refrigeration, the drink kept its acidity stable. Santos Filho (2018) [16], in his article on the influence of temperature in cocoa juice over 42 days, highlights the low variance of organic acids during the study. This highlights the impact of time and temperature on product stability.

Table 2. Result of the physico-chemical analyses in the different storage times of cocoa honey mate tea at room temperature and refrigerated.

Sample	CM1		CM2	
Physical-Chemical Analysis	T0	T45	T0	T45
°Brix (%)	7,9	7,5	8,03	7,4
Titratable Acidity (m/v)	2,83	2,17	2,53	2,5
Vitamin C (mg/100g)	1,37	1,71	1,71	1,03
pH	3,3	3,1	3,24	3,1

At ambient and refrigerated temperature, the pH difference in CM1 and CM2 was minimal, which can be attributed to natural degradation processes or interactions between the ingredients of the prototype. In the study of Koop (2014) [13] with bubble tea based on mate tea and pitanga pulp at room temperature, pH values remained stable for 30 days, also presenting low variations.

With regard to the soluble solids content (°Brix), in CM1 and CM2 there was a reduction. This decrease can be attributed to different physicochemical and microbiological factors. Among the main causes, spontaneous fermentation of simple sugars by yeasts and bacteria naturally present in the product may result in the conversion of these sugars into ethanol and organic acids, directly reducing the °Brix, or action of endogenous enzymes, that promote the hydrolysis of oligosaccharides, altering the profile of soluble sugars (Fellows, 2018; Guirlanda, 2022 [7], [8]).

As for the percentage of vitamin C, there was a reduction in CM1 and an increase in CM2. The increase may have occurred because the analysis has accounted for antioxidant compounds of cocoa honey. The reduction, which normally occurs, was a factor in the sensitivity of vitamin C to storage time. The ideal scenario, which did not occur, would be that this decrease was in CM2, since vitamin C degrades faster at room temperature. Nakilcioğlu-Taş and Ötleş (2020) [14] address in their article on vitamin C and the degradation time in citrus juices, highlighting factors such as storage temperature, light and presence of oxygen influence this loss.

Although present, the identified vitamin C content is below the limit required by legislation for the product to be considered a source of this nutrient. According to the Resolution no 269 of



September 22, 2005 (Brazil, 2005 [5]), the Dietary Reference Intake (DRI) must be 45 mg for adults, and the result is below the recommended. However, it is necessary to consider that the drinks in question are combinations of mate tea with cocoa honey, which interferes with the value found. Then, one possibility is to potentialize them with ingredients that add value to the nutritional profile of this product.

Finally, the sensory analysis was performed with untrained tasters, evaluating the attributes of aroma, color, flavor and texture of teas CM1 and CM2. For both samples, the results showed that the tasters did not notice a significant difference in up to 45 days of conducting the study, presenting an excellent acceptance in all attributes and a promising potential for the market.

4. Conclusion

The cocoa honey mate teas demonstrated that the products maintained their microbiological safety and physico-chemical and sensory stability throughout the period analyzed, indicating that the processing and storage conditions were adequate. We observed a longer conservation in CM2, however, this difference was not very expressive in relation to CM1, especially regarding the microbiological and sensory results, which remained stable.

Certainly, the heat treatment of cocoa honey, and the use of additives and preservatives, contributed to extending the shelf life of cocoa honey tea. The continuity of the study will allow to verify if the stability, quality and shelf life of teas are maintained for a longer period.

Acknowledgement

To SENAI CIMATEC, for the support to the realization of the project, providing its facilities, equipment and a highly qualified technical team. To COOPERMATA, for partnership and direct collaboration in the development of activities. To EMBRAPPI and SEBRAE, for institutional support and encouragement of innovation.

References

- [1] Adolfo Lutz Institute. Normas analíticas do Instituto Adolfo Lutz: métodos físico-químicos para análises de alimentos. 4ª ed. 1ª ed. digital. São Paulo: Instituto Adolfo Lutz; 2008. 1020 p. Available from: <https://wp.ufpel.edu.br/nutricaoobromatologia/files/2013/07/NormasADOLFOLUTZ.pdf>.
- [2] Brazil. Agência Nacional de Vigilância Sanitária. Resolution RDC No. 724, of 29 September 2022. Diário Oficial da União. Brasília (DF); 2022 Sep 30.
- [3] Brazil. Ministério da Saúde. Normative Instruction No. 161, of 17 August 2021. Diário Oficial da União. Brasília (DF); 2021 Aug 18.
- [4] Brazil. Ministério da Saúde. Portaria nº 519, de 26 de junho de 1998. Aprova o Regulamento Técnico para fixação de identidade e qualidade de produtos vegetais, partes de vegetais e misturas de vegetais destinados ao preparo de infusões e/ou decocções. *Diário Oficial da União: seção 1*, Brasília, DF, n. 122, p. 10, 30 jun. 1998.
- [5] Brazil. Resolução nº 269, de 22 de setembro de 2005. Dispõe sobre a Ingestão Diária Recomendada de vitaminas e minerais para a população brasileira. Diário Oficial da União, Brasília, DF, 27 set. 2005.



- [6] Brazilian Association of Technical Standards. ABNT NBR ISO 11136:2022. Análise sensorial – Metodologia: guia geral para condução de testes hedônicos com consumidores em ambientes controlados. Rio de Janeiro: ABNT; 2022.
- [7] Fellows, P. J. *Tecnologia do processamento de alimentos: princípios e prática*. 4. ed. Porto Alegre: Artmed, 2018.
- [8] Guirlanda CP, et al. Characterization, processing study and preservation of cocoa honey [thesis]. Belo Horizonte (MG): Universidade Federal de Minas Gerais; 2022.
- [9] Guirlanda CP, da Silva GG, Takahashi JA. Cocoa honey: agro-industrial waste or underutilized cocoa by-product? *Future Foods*. 2021;4:100061.
- [10] Grizotto RK, et al. Shelf-life study of structured and dehydrated fruit obtained from concentrated papaya pulp. *Ciênc Tecnol Aliment*. 2006;26(3):709–14.
- [11] IBGE – Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2017–2018: análise do consumo alimentar pessoal no Brasil. Rio de Janeiro: IBGE, 2020.
- [12] International Cocoa Organization. Quarterly Bulletin of Cocoa Statistics – May 2025 [Internet]. Abidjan: ICCO; 2025 [cited 2025 Aug 1]. Available from: <https://www.icco.org/may-2025-quarterly-bulletin-of-cocoa-statistics/>.
- [13] Koop BL, et al. Development of bubble tea-type beverage based on mate tea with native fruit pulp and added nectar spheres of the same fruit [undergraduate monograph]. Laranjeiras do Sul (PR): Universidade Federal da Fronteira Sul; 2017.
- [14] Nakilcioğlu-Taş E, Ötleş S. Kinetic modelling of vitamin C losses in fresh citrus juices under different storage conditions. *An Acad Bras Cienc*. 2020;92(1):e20190328. doi:10.1590/0001-3765202020190328.
- [15] Pereira, Tafaél Lucas, Braga, Ana Carolina. Review of good manufacturing practices in the university dining restaurant process. *Revista Espacios*. 2014;35(5).
- [16] Santos Filho LP. Physical-chemical and microbiological evaluation of fermented cocoa juice with *Lactobacillus casei* [master's dissertation]. São Luís (MA): Universidade Federal do Maranhão; 2018.