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# Kombucha-Based Cocoa Honey Beverage

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**Abstract:** The objective of this study was to produce kombucha beverage using cocoa honey as substrate and to characterize it through physicochemical, microbiological and sensory analysis, in order to compare these parameters with those obtained through pure cocoa honey analysis and commercial kombucha. The results show that the characteristics obtained in kombucha-based cocoa honey beverage are favorable for future commercialization. The new beverage presented low acidity and presented high sensory acceptance compared to the commercial kombucha analyzed.

**Key words:** Food with functional potential · Fermentation · Scoby (Symbiotic culture of bacteria and yeast)

## INTRODUCTION

Kombuchais a sweet, slightly acidic beverage produced by fermenting tea with added sugar by a symbiotic culture known as scoby (Symbiotic Culture of Bacteria and Yeast) [1]. Kombucha beverage is mainly composed of yeast and acetic acid bacteria in addition to lactic acid bacteria in smaller proportions. These microorganisms, through the fermentation process, generate metabolites such as amino acids, polyphenols, organicacids, vitamins, micro elements and antibiotics, which give the kombucha an antioxidant, anti-hyperlipidemic, anti-hyperglycemic, antimicrobial and anticarcinogenic effect [2, 3]. In 2017, the commercial kombucha became the product with the highest growth in sales in the functional beverage smarket. The Kombucha beverage sales increased 37.4% due to the increase in its popularity among low alcohol fermented beverages [4]. Currently industrialized kombucha is sold in commercial establishments and can be found in several flavors (fruits and herbal tea) [5]. Due to the popularization of Kombucha and the beneficial effects of its consumption, the search for different substrates for the production of the Kombucha beverage has grown [6]. For the fermentation process to occurs at is factorily, these substrates must contain sources of carbon and nitrogen [7].

Cocoa (*Theobroma cacao* L.) is widely produced and marketed by tropical and subtropical countries, among which Brazil stands out [8]. The nutritional value of cocoa contributed to the consolidation of its national and international consumption. Cacao is used mainly for the production of chocolate, but it is also capable of producing "cocoa honey" [9].

Cocoa honey is a mucilaginous liquid released from the pulp that surrounds the cocoa beans immediately before fermentation during the production process. Its flavor varies between sweet and sour, has a high content of reducing sugars and a significant amount of fiber. In addition to being considered a natural source of bioactive phenolic compounds and antioxidants, cocoa honey has good potential, still under-explored, for technological applications in the food industry [10].

A variety of substrates have been previously tested for the production of kombucha, including some dairy products, fruits, vegetables, herbs, coconut water and coffee [3]. However, there are no reports on the use of cocoa honey for the production of kombucha. Thus, the

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aim of this study was to produce kombucha using cocoa honey as a fermentation substrate. In addition to characterizing Kombucha-based cocoa honey beverage through physical-chemical, microbiological and sensory analysis. The data obtained were compared with parameters of the analysis of pure cocoahoney and commercial kombucha.

## MATERIALS AND METHODS

**Production of Kombucha-Based Cocoa Honey Beverage:** The kombucha was prepared by fermentation in green tea. The green tea extract was produced with 6.4 g of *Camellia sinensis* leaves to 1 liter of water for 10 minutes in infusion and 50 g of demerara sugar was added and the solution was stirred until dissolved. After reducing the temperature of green tea (around 28°C), the scoby-daughter was inoculated at a rate of 10% (Fig. 1A, B, C, D). The scoby Kombucha was provided by the Food Microbiology Laboratory of the Faculty of Pharmacy of the Federal University of Bahia (UFBA). This procedure was previously described by Suciati *et al.* [11]. Fermentation was carried out for 4 days using the artisanal method: a 3-liter glass container with an opening of 9 cm in diameter, covered with paper towels, which allows air to pass through without contamination by insects or other physical dirt (Fig. 1 E). Cocoa honey (purchased from the city of Salvador) was added to this starter culture at a 9: 1 ratio for the second fermentation (Fig. 1F), for 2 days at 28°C, in a Polyethylene Terephthalate (PET) container. Subsequently, the contents of the second fermentation were stored at a temperature of 4°C for 1 day, to perform physical-chemical, microbiological and sensory analyzes.

**Physicochemical Analysis:** The physical-chemical evaluation was carried out at the Food Microbiology Laboratory of the UFBA Faculty of Pharmacy in Salvador / BA. The analyzes of total acidity, pH and total soluble solids (°Brix) were performed in triplicate. The methodology described in the Adolfo Lutz Institute's manual of physical-chemical methods for food analysis was used [12]:

**Total Acidity:** The total acidity was determined by neutralizing the sample with sodium hydroxide (NaOH) 0.1 mol $L^{-1}$. 5 mL of the sample was pipetted into a 125 mL conical flask. 50 ml of distilled water and 3 drops of 1% phenolphthalein solution were added. Then the titration was started with the 0.1 mol.$L^{-1}$ NaOH solution until it reached the turning point (pink color). Acidity was calculated using the equation:

$$n \times M \times f \times PM / 10 \times V = \text{total acids, in grams of acetic acid per 100 mL of sample}$$

where:

- n = Volume used for the titration of the sodium hydroxide solution, in mL
- M = Molarity of sodium hydroxide solution
- f = Correction factor for sodium hydroxide solution
- PM = Molecular weight of acetic acid (60g)
- c = Used volume of the sample, in mL

**pH:** The pH was determined by potentiometry, dipping the electrode directly into the sample and recording the pH value indicated on the pH meter.

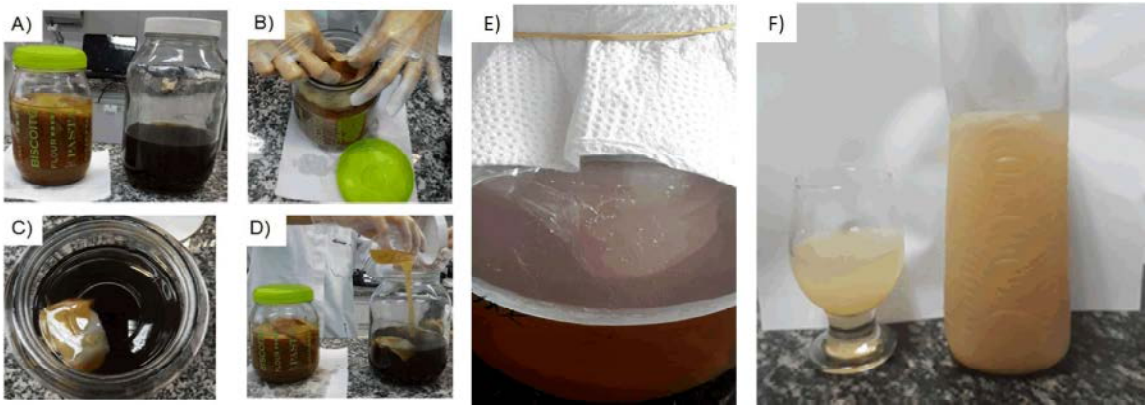


Fig. 1: Preparation of Kombucha: A) Scoby and green tea used. B) Scoby to incorporate into the green tea produced. C) Inoculum in green tea. D) 10% scoby-daughter inoculum E) First stage of Kombucha fermentation. F) Second stage of Kombucha fermentation with cocoa honey

**Total Soluble Solids (°Brix):** The total soluble solids were determined by refractometric method through direct reading on the manual refractometer. Two drops of the sample were transferred to the refractometer prism. After closing the prism, the reading was performed observing the internal scale of the device. The results were expressed in (°Brix).

**Microbiological Analysis:** Analyzes of molds, yeasts, acetic acid bacteria and lactic acid bacteria were carried out using the methodology described in the Microbiological Food Analysis Methods Manual [13]. For the analysis, the samples were diluted, aseptically removing 1 mL of the sample and adding 9 mL of sterile 0.1% peptoned water ($10^{-1}$ dilution). From that aliquot, subsequent serial dilutions were performed until the $10^{-8}$ dilution was reached. Microbiological analyzes were also performed at the Food Microbiology Laboratory of the UFBA Faculty of Pharmacy in Salvador / BA.

**Molds and Yeasts:** Mold and yeast counting was performed by inoculating 0.1 mL of each dilution in plates containing Dichloran Rosa Bengal Chloramphenicol agar (DRBC), spreading with the drigalski loop until the aliquot is fully absorbed. The plates were incubated for a period of five days at 25°C and those with typical colony growth were selected for counting. The results were analyzed and expressed in CFU/mL.

**Acetic Acid Bacterias:** The counting of acetic acid bacteria was carried out by inoculating 0.1 mL of each dilution in plates containing Glucose Yeast Maltose agar (GYM), spreading with the drigalski loop until the excess liquid was absorbed. The plates were incubated at 35°C for 48 hours and those that showed growth of typical colonies were selected for counting. The results were analyzed and expressed in CFU/mL.

**Lactic Acid Bacterias:** The counting of lactic acid bacteria was performed by inoculating 0.1 mL of each dilution in plates containing Agar De Man Rogosa and Sharpe (MRS), spreading with the drigalski loop until the excess liquid was absorbed and incubated at 35°C for 48 hours. Plates that showed typical colony growth were selected for counting. The results were analyzed and expressed in CFU/mL.

**Sensory Analysis:** Sensory tests were performed in a sensory analysis laboratory at UFBA Faculty of Pharmacy in Salvador / BA, under controlled conditions. The samples of kombucha-based cocoa honey beverage (KCH), pure cocoa honey (CH) and commercial kombucha tangerine flavor (KCT) were sensorially analyzed for acceptance and purchase intention, with the participation of 100 untrained volunteers (tasters), without restriction as to age, sex or social class. Initially the tasters participated in a survey, answering a questionnaire about the kombucha product in general (Do you know Kombucha?; Do you consume or consumed Kombucha?; Why did you start consuming Kombucha?; Do you know the benefits of Kombucha?).

The three previously coded samples were analyzed monadically, where each taster received approximately 20 mL of each chilled drink (KCH, CH and KCT), served in a disposable cup. The acceptance test was performed using the parameters of appearance, color, aroma, texture, flavor and general acceptance, through the structured hedonic scale of nine points, being 9 = very much liked and 1 = very disliked [14]. The results obtained were analyzed statistically by Analysis of Variance (ANOVA), using the Tukey test, with a reliable level of 95% for each parameter reached, using the Minitab 17 program. The data will be presented in the configuration: mean ± standard deviation.

The purchase intention test was carried out in a similar way, where the tasters needed to mark the chosen purchase option with "X". The options offered were: I would certainly buy; would probably buy; I have doubts if I would buy this product or not; probably would not buy and; certainly would not buy. To construct the purchase intention graph and read the results, the options questioned were subsequently represented using the five-point structured scale, with 5 = certainly buying and 1 = certainly not buying [14].

## RESULTS AND DISCUSSION

The data from the analyzes carried out on the kombucha sample produced with green tea and cocoa honey (kombucha-based cocoa honey beverage) were evaluated in order to compare the interaction between the physical-chemical and microbiological results after obtaining the developed product. In this way, the sample of the elaborated product was compared to a commercial tangerine flavored Kombucha and to pure cocoa honey, both purchased in local stores in the city of Salvador (BA). The results were standardized by relating the data obtained in the analysis of pH, total acidity and total soluble solids content (°Brix), as shown in Table 1. The microbiological results were also used (Table 2). All analyzes were performed after the end of the second fermentation.

Table 1: Average values of the physical-chemical parameters (total acidity, pH and total soluble solids content (°Brix)

| | KCH | KCT | CH |
|---|---|---|---|
| Total acidity(%) | $1.12^{a} \pm 0.00$ | $0.64^{b} \pm 0,01$ | $1.28^{c} \pm 0.01$ |
| pH | $3.51^{a} \pm 0,21$ | $3.45^{a} \pm 0,04$ | $3.49^{a} \pm 0.01$ |
| °Brix | $14.03^{a} \pm 0,06$ | $6.00^{b} \pm 0,00$ | $19.23^{c} \pm 0.06$ |

KCH: Kombucha-based cocoa honey beverage, KCT: Commercial kombucha tangerine flavor, CH: Pure cocoa honey. Triplicate mean ± standard deviation. Different letters on the same line: significant differences ($p < 0.05$)

Table 2: Count of molds and yeasts, acetic acid bacteria and lactic acid bacteria, in KCH: Kombucha-based cocoa honey beverage, KCT: Commercial kombucha tangerine flavor, CH: Pure cocoa honey

| | Molds and Yeasts (CFU/mL) | Acetic Acid Bacteria (CFU/mL) | Lactic acid bacteria (CFU/mL) |
|---|---|---|---|
| KCH | $1.0 \times 10^{8}$ | >300 | <10 |
| KCT | $1.0 \times 10^{7}$ | >300 | <10 |
| CH | $1.0 \times 10^{7}$ | > 300 | $1.0 \times 10^{5}$ |

The pH analyzes were performed on a pH meter, after the samples were removed. The analyzes of artisanal Kombucha-based cocoa honey beverage were performed in triplicate, with no significant differences ($p < 0.05$) in their results. The Kombucha-based cocoa honey beverage showed pH 3.51, after 6 days of fermentation, while for purê cocoa honey, pH 3.49 was detected, similar to the value found by Leite *et al.* [16], pH 3.51. The low pH value is attributed to the production of various organic acids during fermentation, with the main organic acids found, acetic acid and gluconic acid [16]. According to Normative Instruction No. 41 of September 17, 2019 [17], the pH of the kombucha should have values between 2.5 and 4.2, with minimum and maximum levels, respectively.

Regarding the content of total soluble solids, the CH sample indicated a higher value of °Brix (19.23), followed by the KCH and KCT samples, with 14.03 and 6.00 respectively. The two samples of kombucha (KCH and KCT) showed a significant distinction as to °Brix, due to the raw material used, since the pure cocoa honey has a high content of total soluble solids. For the total soluble solids contente. Leite *et al.* [16] found for its sample of cocoa honey the value of 13.30 °Brix, a value below that found in the cocoa honey used in this analysis. This value can be variable due to the handling and production of cocoa honey, as well as the production of acids during the fermentation process, which also justifies the reduction of the degree of total soluble solids, since the yeasts and acetic bacteria present in the scoby consume the substrate sugar, as discussed by Santos *et al.* [18].

Total acidity was also different between samples, being 0.64; 1.12 and 1.28 for the KCT, KCH and CH samples respectively. This result is directly linked to what was previously exposed in relation to the content of total soluble solids and pH. From the evaluated parameters, it was observed that the KCH sample has a less acidic and slightly sweet characteristic when compared to the commercial kombucha tangerine flavor (KCT).

According to Table 2, it is possible to observe the presence of $1.0 \times 10^{8}$ CFU/mL of molds and yeasts in the KCH sample and $1.0 \times 10^{7}$ CFU/mL in the KCT and CH samples, as well as the presence of acetic bacteria (> 300 CFU/mL) in all samples. A lower amount was also observed in relation to lactic acid bacteria in KCH and KCT kombuchas. This fact is due to the characteristic of high acidity that the beverage has due to the fermentation process performed by the acetic bacteria, which are prevalent in kombucha [19]. It is also important to highlight the symbiotic relationship between yeasts and acetic bacteria that occurs due to the production of ethanol by the yeasts and the consequent consumption of this by the acetic acid bacteria, causing acidification of the medium. While the growth of bacteria is stimulated by the production of growth factors (vitamins) and nitrogen compounds soluble in yeasts. In contrast, according to Schwan [20], cocoa honey has lactic acid bacteria, mostly *Lactobacillus fermentum* and *Lactobacillus plantarum*. These species develop through cocoa fermentation and consume the sugars in the pulp before the honey develops.

The sensory analysis showed that among the 100 tasters, aged between 23 and 51 years old, 68 were female. According to the answers obtained by the questionnaire applied (Fig. 2), 77 tasters already knew or heard about the kombucha beverage and 71 knew how to indicate some benefit provided by Kombucha. Among the tasters, only 9 consumed kombucha monthly, while 33 rarely and 58 never. When asked why they started consuming the kombucha beverage, the tasters also indicated the options "health benefits" and "food trends".

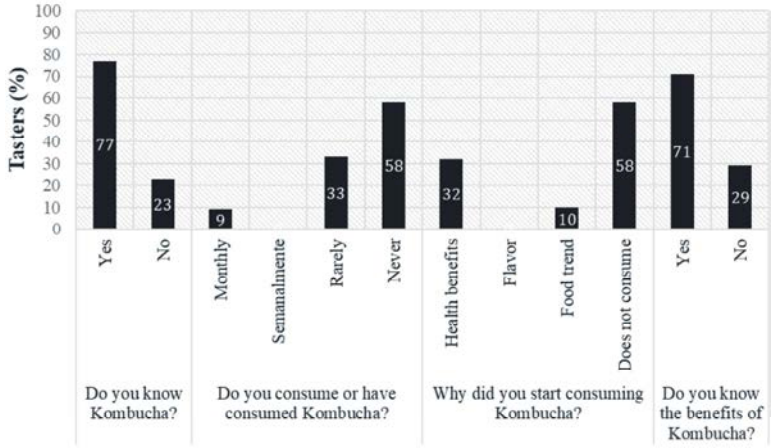


Fig. 2: Distribution of responses obtained through the kombucha question naire

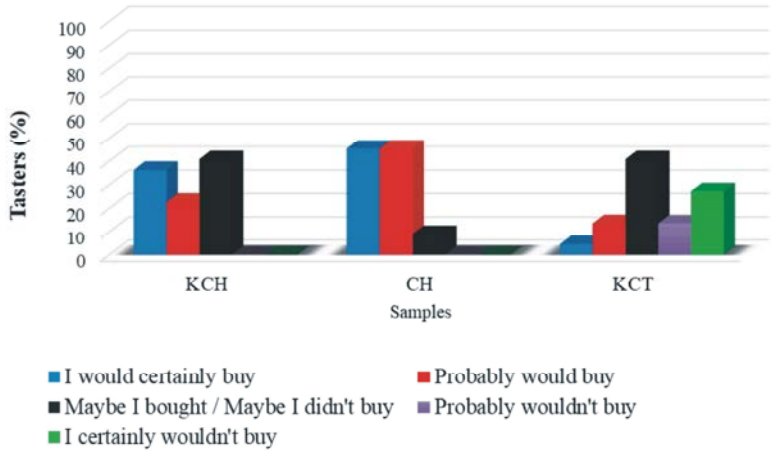


Fig. 3: Distribution of frequency for the test of purchase intention of samples of KCH: Kombucha-based cocoa honey beverage, KCT: Commercial kombucha tangerine flavor, CH: Pure cocoa honey

Table 3: Response variables of sensory acceptance tests

| | Response Variables | | | | |
|---|---|---|---|---|---|
| | Appearance | Aroma | Flavor | Texture | GA |
| KCH | 7.32$^{a}$ ± 1.3588 | 6.55$^{a}$ ± 1.4385 | 6.91$^{a}$ ± 1,7433 | 7.36$^{a}$ ± 1.5289 | 7.14$^{a}$ ± 1.1668 |
| CH | 7.35$^{a}$ ± 1.6488 | 7.00$^{a}$ ± 1.6330 | 8.00$^{a}$ ± 1.0235 | 7.91$^{a}$ ± 1.1088 | 7.95$^{a}$ ± 0.7854 |
| KCT | 7.40$^{a}$ ± 1.3355 | 5.86$^{a,b}$ ± 1.8592 | 4.68$^{b}$ ± 2.1687 | 6.95$^{a}$ ± 1.3266 | 5.27$^{a,b}$ ± 1.9069 |

GA: Global acceptance; KCH: Kombucha-based cocoa honey beverage, KCT: Commercial kombucha tangerine flavor, CH: Pure cocoa honey. Mean (n.22) ± standard deviation. The means followed by the same letter in a column do not differ significantly (Tukey's test; p> 0.05).

The results of the acceptance test are shown in Table 3. The sample of pure cocoa honey (CH), presented higher scores attributed to the parameters of appearance, aroma, flavor, texture and overall impression, being 7.35; 7.00; 8.00; 7.91 and 7.95 respectively. The Kombucha-based cocoa honey beverage (KCH) showed notes similar to pure cocoa honey, while the commercial kombucha tangerine flavor sample (KCT) indicated lower scores in relation to the other samples, except for the appearance parameter, which indicated top note of 7.40. The KCT sample also indicated a significant difference (p> 0.05) for the aroma, flavor and overall impression parameters, whereas the KCH and CH samples did not differ significantly (p <0.05) between the analyzed parameters.

Fig. 3 shows that the sample of pure cocoa honey (CH) obtained the highest positive purchase intention (45%), followed by the sample Kombucha-based cocoa honey beverage (KCH) with 36%.

This result was already expected due to the sweet and refreshing taste of cocoa honey. However, it was found that most of the tasters were still in doubt as to the purchase of both samples, indicating 45% and 23% in the category "maybe bought / maybe not bought" for samples CH and KCH respectively. KCT presented 41% for the same category, while only 5% of tasters claimed that they would certainly buy such a beverage. The results also show that both pure cocoa honey and the kombucha beverage produced from it (KCH), showed positive acceptance in relation to the third sample of commercial kombucha tangerine flavor. This result is due to the fact that both have a higher degree of soluble solids than the KCT sample, while the values of total acidity and pH remained similar. The amount of soluble solids evaluated can directly interfere in the acceptance of the analyzed samples and, preferably, in the developed product.

Other authors have also found favorable results in the preparation of different foods with an increase in nutritional and sensory characteristics: Hassan *et al*. [21] confirms that lycopene which extracted from tomato wastes can be used in preparing processed cheese with high content of iron, antioxidant and phenolic compounds. Ali *et al*. [22] show in a in vivo study, six groups of rats fed for 14 days with either plain yoghurt or Aloe vera (AV) supplemented yoghurt (10% and 20%). Serum protein and lipid profiles were assessed. Results from in vivo studies showed a noticeable increase in weight gain, total proteins and globulins over a period of 14 days in experimental animals fed AV yoghurt. Ghazali *et al*. [23] performed clinical work among six students from Malaysian primary schools. The purpose of the clinical work was to see whether private class, parenting advice and food supplements, which were provided for the chosen students, can give a significant different in learning disabled students before and after the clinical work. Assessing by the test scores of Malay language and Mathematics, the clinical work was a success due to the learning disabled students able to show significant improvement before and after the clinical work. Hence, private class, parenting advices and food supplement can be used as the rehabilitation tools for learning disabled students in helping them to learn well as their normal peer.

## CONCLUSION

The kombucha, although not yet widely recognized, has been gaining space in the Brazilian market due to its health benefits. The results showed that cocoa honey can be used as an alternative substrate for Kombucha production. The characteristics obtained for Kombucha-based cocoa honey beverage are favorable for its possible commercialization, since the beverage presented a high positive sensory acceptance compared to the commercial kombucha analyzed.

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