EVALUATION OF KOMBUCHA PRODUCTION FROM GREEN TEA (*Camellia sinensis*) FLAVORED WITH COCOA BEAN SHELL (*Theobroma cacao L.*)

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ABSTRACT

Kombucha is a functional drink with added value and consumed worldwide, made with *Camellia sinensis* tea, sugar, and the addition of SCOBY (symbiotic colony of bacteria and yeast), responsible for fermentation. It can be enriched with fruits, spices, and other ingredients to diversify its flavor and add benefits. In contrast, the shell of *Theobroma cacao L.* almonds (CBS), a byproduct of the cocoa industry, is rich in bioactive compounds such as polyphenols and antioxidants. The objective of this study is, to convert this byproduct and compare traditional kombucha (K0) with kombuchas flavored with 0.1% (K1) and 0.5% (K2) of cocoa bean shell (CBS). The production followed the Brazilian quality standards established by MAPA. In relation to bioactive compounds, kombucha K2 showed higher results than K0 and K1, with concentrations of polyphenols (mEq.Ac.Gallic/100g) 126.23 (K0) and 170.89 (K2), flavanols (mEq.Catechin/100g) 11.95 (K0) and 30.48 (K2), flavonols (mEq.Rutin/L) 24.03 (K0) and 53.81 (K2), and antioxidants (µmol.Eq.Trolox/g) 4.79 (K1) and 6.93 (K2). Therefore, kombucha with a higher concentration of cocoa bean shell demonstrated more potential antioxidant benefits present in the functional fermented drink.

Keywords: Kombucha. Cocoa. Characterization. Antioxidants.

1 INTRODUCTION

Kombucha is known as a functional beverage with a slightly sweet and acidic flavor that contains significant therapeutic properties[1]. It is made through a process that involves both aerobic respiration and anaerobic fermentation, using a wort resulting from the infusion or extract of *Camellia sinensis*, added with sugars through a symbiotic culture of microbiologically active bacteria and yeasts, known as SCOBY[2]. The process is divided into two fermentations; in the first stage, it covers a period of 3 to 60 days in which the yeasts ferment the sugar and produce ethanol while the bacteria oxidize the alcohol to produce acetic acid[3]. Other organic acids are also formed, which have antibacterial activity and a new biofilm (SCOBY), obtaining the original kombucha composed only of the essential ingredients. Due to the microbial fermentations of kombucha, they promote the increase of its bioactive properties, in addition to triggering numerous benefits attributed to the presence of probiotic microorganisms (acetic and lactic acid bacteria), amino acids, polyphenols, vitamins, and a variety of micronutrients[4]. In the second stage, products are added for flavoring that will bring sensory enhancement and antioxidant phenolic compounds. At this stage, products such as seasonings, spices, fruits, or medicinal herbs are added, elevating the taste experience, and improving the intrinsic benefits of the beverage. Cocoa (*Theobroma cacao L.*) is a food rich in bioactive compounds of great interest to the food, cosmetic, and pharmaceutical industries[5]. These phenolic compounds are stored in the cotyledons of cocoa seeds, and during fermentation, the loss of these compounds occurs due to diffusion out of the cotyledon. As a result, the cocoa bean shell becomes a material rich in such bioactive compounds[6], being a residue that contains 10 to 12% of the dry weight of the almond that is not used by the industry, but studies reveal that it contains phenolic compounds[5]. The objective of this work is to enhance the therapeutic benefits of kombucha with the addition of a cocoa byproduct (*Theobroma cacao L.*), the cocoa bean shell, and the valorization of this byproduct.

2 MATERIAL & METHODS

Two kombuchas were prepared, the first kombucha is the original one with green tea leaves (*Camellia sinensis*), which was obtained from the market in Belém (State of Pará, Brazil), and the second kombucha was flavored with cocoa bean shell (*Theobroma cacao L.*), analyzed based on the concentration of phenolic compounds[10,11]. At the beginning of the process, the first fermentation was prepared with 10g of green tea leaves per 1 liter of water with the addition of 100g of sugar for the infusion at 90°C for 15 minutes. Then, it was filtered and divided into two smaller volumes of 500 mL to be transferred into two previously sterilized glass containers. Subsequently, 10% (v/v) of kombucha as a liquid starter and the symbiotic culture of bacteria and yeasts (SCOBY), provided by students from the Federal Rural University of the Amazon, were added to the green tea. The jars were covered with cotton fabric and stored under light shelter for fermentation for 10 days at room temperature. For the second fermentation, the kombucha was filtered to remove the SCOBY and added with 0.4g and 2g of cocoa bean shell, which were analyzed based on the presence of phenolic compounds (Table 2), corresponding to 0.1% and 0.5% in relation to the total volume of 400 mL, respectively. After 7 days of flavoring, the kombuchas were filtered, bottled, and kept refrigerated. During the production of the fermented beverage, aliquots of the original kombucha were taken during the first 10 days of fermentation, for pH measurement, total soluble solids (TSS) in degree Brix, and total acidity[7], aiming to monitor the

fermentation process. In addition, analyses of TP (total polyphenols)[8], DPPH (2,2-diphenyl-1-picrylhydrazyl)[9], TFO (Total Flavonols)[10], and TFA (Total Flavanols)[11] were performed on the final aliquots to determine the phenolic and antioxidant compounds. For the kombuchas containing cocoa bean shell, all analyses were performed at the end of the flavoring.

3 RESULTS & DISCUSSION

In the comparison of the results obtained for the original kombucha (K0) and kombuchas flavored with 0.1% (K1) and 0.5% (K2) of cocoa bean shell (CBS), parameters from Normative Instruction (NI) No. 41 of 2019[2], established by the Ministry of Agriculture, Livestock, and Supply (MAPA), were used. The minimum and maximum values of pH, total acidity (mEq/L), and total soluble solids (ºBrix) for the samples varied according to Table 1, demonstrating compliance with the quality standard established by MAPA.

Table 1 Comparison of pH, Total Acidity, and Soluble Solids parameters of original Kombucha (K0) and flavored with 0.1% (K1) and 0.5% (K2) of cocoa bean shell.

	pH	TA(mEq/L)	TSS (ºBrix)
Legislation	2.5 - 4.2	30 - 130	-
K0	3.18 ± 0.07	78.80 ± 0.001	9.65 ± 0.08
K1	2.95 ± 0.006	120.18 ± 0.004	9.37 ± 0.15
K2	3.11 ± 0.006	100.8 ± 0.003	9.83 ± 0.05

Legend: Total Acidity (TA), Total Soluble Solids (TSS).

In relation to the phenolic compounds and free radical scavenging activity of the kombuchas, these compounds increase in total quantities due to the degradation of larger compounds into smaller ones by the action of enzymes produced and released by the SCOBY[12]. There was a significant increase in the analyses, with the presence of 170.79 (mEq.Ac.Gallic/100g) of total polyphenols (TP), 30.48 (mEq.Catechin/100g) of flavanols (TFA), 53.81 (mEq.Rutin/L) of flavonols (TFO), and 6.93 (µmol.Eq.Trolox/g) of DPPH in the kombucha flavored with 0.5% of cocoa bean shell (K2). In the kombucha flavored with 0.1%, the results were lower, but still obtained enhanced properties compared to traditional kombucha in the analyses of total polyphenols (TP), total flavonols (TFO) and antioxidant compounds (DPPH) (Table 2). The increase in concentrations of bioactive compounds can be attributed to the high content of flavonols and other polyphenols, especially catechins and epicatechins, methylxanthines such as theobromine and caffeine, present in the composition of Theobroma cacao L. and byproducts such as cocoa bean shell (CBS)[13].

Table 2 Analysis of Phenolic Compounds of original Kombucha (K0) and flavored with 0.1% (K1) and 0.5% (K2) of cocoa bean shell (CBS).

	TP (mEq.Gallic Acid/100g)	TFA (mEq.Catechin/100g)	TFO (mEq.Rutin/L)	DPPH (µmol.Eq.Trolox/g)
CBS	449.13 ± 0.01	38.46 ± 1.54	44.06 ± 2.64	1.12 ± 3.62
K0	126.23 ± 0.005	11.95 ± 0.53	24.03 ± 0.63	4.79 ± 0.33
K1	136.22 ± 0.005	11.22 ± 0.36	26.10 ± 0.43	6.02 ± 0.56
K2	170.79 ± 0.004	30.48 ± 0.59	53.81 ± 1.58	6.93 ± 0.79

Legend: Total Polyphenols (TP), Total Flavanols (TFA), Total Flavonols (TFO), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

4 CONCLUSION

Fermented beverages based on *Camellia sinensis* tea, both traditionally made and with the addition of percentages of cocoa bean shell (*Theobroma cacao L.*), demonstrate results in conformity with Brazilian quality standards established by Normative Instruction No. 41 of the Ministry of Agriculture, Livestock, and Supply (MAPA). However, kombuchas flavored with 0.1% and 0.5% of cocoa bean shell revealed a higher pH and acidity compared to traditional kombucha, resulting in flavor differentiations. Additionally, both variants showed an increase in the concentration of phenolic compounds and inhibition of free radicals, especially in the formulation with a higher concentration of cocoa byproduct, indicating an excellent alternative for the reuse of this waste and also for obtaining a fermented beverage with intensified functional aspects.

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