



ELABORAÇÃO E CARACTERIZAÇÃO FÍSICO-QUÍMICA DE *COLD BREW* FERMENTADO A PARTIR DE CULTURA SIMBIÓTICA DE KOMBUCHA

ELABORACIÓN Y CARACTERIZACIÓN FÍSICOQUÍMICA DE *COLD BREW* FERMENTADO A PARTIR DE UN CULTIVO SIMBIÓTICO DE KOMBUCHA

ELABORATION AND PHYSICOCHEMICAL CHARACTERIZATION OF FERMENTED *COLD BREW* USING A KOMBUCHA SYMBIOTIC CULTURE

Apresentação: Comunicação Oral

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DOI: <https://doi.org/10.31692/VIICIAGRO.0053>

RESUMO

A kombucha, bebida elaborada pela fermentação simbiótica de chá, tem apresentado visibilidade e elevado consumo devido às suas propriedades funcionais e potencial probiótico. Na busca por inovação de aromas e sabores, o café pode ser uma excelente base para essa bebida, e o método de extração a frio do café (*cold brew*) tem se destacado pela refrescância, menor amargor, e aromas e sabores específicos. Diante do exposto, o objetivo deste trabalho foi desenvolver e caracterizar físico-quimicamente *cold brew* 100% arábica fermentado a partir da cultura simbiótica de kombucha. Foram elaboradas bebidas com diferentes concentrações de *cold brew* em chá verde (25, 45, 65 e 90% v/v), sendo acompanhada a fermentação pelas análises de teor de sólidos solúveis totais, pH e acidez total titulável, nos tempos 9, 15, 23 e 30 dias. Para a caracterização físico-química das bebidas foram realizadas as seguintes análises: concentração de açúcares redutores, teor de compostos fenólicos, e cor. Durante a avaliação do processo fermentativo houve o aumento do teor de sólidos solúveis totais e acidez total, e redução do pH que obteve valores finais aceitáveis para os limites estabelecidos pela Instrução Normativa N°41/2019. Nos açúcares redutores das amostras fermentadas a variação foi de 21,49 g/100mL a 37,39 g/100mL. Os maiores valores de pH, acidez e açúcares redutores foram encontrados na amostra elaborada com 90% *cold brew*. Todas as bebidas fermentadas apresentaram diminuição significativa ($p < 0,05$) no teor de polifenóis totais em comparação com o *cold brew* não fermentado. Para os demais compostos fenólicos houve um aumento após a fermentação. Porém, entre as amostras com maior concentração de *cold brew*, os teores de flavonoides, flavonóis e antocianinas totais foi superior ao observado para as amostras com maior volume de chá verde. Os taninos totais foram significativamente maiores ($p < 0,05$) apenas nas amostras com 45%v/v de *cold brew* (41,31 mg/100mL) e 90% de *cold brew* (59,77 mg/100mL) em comparação com *cold brew* não fermentado (31,92 mg/100mL). Na coloração, as bebidas fermentadas apresentam cor vermelho-amarelada, luminosidade média, baixa saturação e tonalidade com nuances de vermelho. A diferença cromática só seria percebida, em uma avaliação sensorial, entre a amostra com 25% v/v de *cold brew* e as amostras com 65 e 90% v/v de *cold brew* e o *cold brew* não fermentado. Os resultados mostram que há uma viabilidade do uso do *cold brew* como substrato para kombucha, tornando-se uma alternativa a substituto do chá verde tradicionalmente utilizado.

Palavras-Chave: Bebida fermentada, Cultura Simbiótica, *Cold brew*, Caracterização.

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RESUMEN

La kombucha, bebida obtenida mediante la fermentación simbiótica del té, ha adquirido creciente relevancia y aceptación por parte de los consumidores debido a sus propiedades funcionales y potencial probiótico. En la búsqueda de innovación en perfiles aromáticos y sensoriales, el café constituye un sustrato prometedor para la producción de kombucha, mientras que el método de extracción en frío del café (*cold brew*) ha despertado interés debido a sus características refrescantes, menor amargor y atributos sensoriales distintivos. En este contexto, el objetivo del presente estudio fue desarrollar y caracterizar fisicoquímicamente una bebida fermentada de *cold brew* 100% arábica obtenida mediante el uso de un cultivo simbiótico de kombucha. Se formularon bebidas con diferentes proporciones de *cold brew* y té verde (25, 45, 65 y 90% v/v), y el proceso fermentativo fue monitoreado mediante análisis de sólidos solubles totales, pH y acidez total titulable a los 9, 15, 23 y 30 días de fermentación. La caracterización fisicoquímica incluyó la determinación de azúcares reductores, compuestos fenólicos y parámetros de color. Durante la fermentación se observaron incrementos en el contenido de sólidos solubles totales y en la acidez total titulable, acompañados de una reducción en los valores de pH, cuyos valores finales se mantuvieron dentro de los límites aceptables establecidos por la Instrucción Normativa N° 41/2019. El contenido de azúcares reductores de las muestras fermentadas osciló entre 21,49 y 37,39 g/100 mL. La bebida formulada con 90% de *cold brew* presentó los mayores valores de pH, acidez y azúcares reductores. Todas las bebidas fermentadas mostraron una disminución significativa ($p < 0,05$) en el contenido de polifenoles totales en comparación con el *cold brew* no fermentado. Por el contrario, las concentraciones de otros compuestos fenólicos aumentaron tras la fermentación. Entre las formulaciones con mayor concentración de *cold brew*, los contenidos de flavonoides, flavonoles y antocianinas totales fueron superiores a los observados en las bebidas con mayor proporción de té verde. El contenido de taninos totales fue significativamente mayor ($p < 0,05$) únicamente en las bebidas con 45% v/v de *cold brew* (41,31 mg/100 mL) y 90% de *cold brew* (59,77 mg/100 mL) en comparación con el *cold brew* no fermentado (31,92 mg/100 mL). En relación con los parámetros de color, las bebidas fermentadas presentaron coloración rojo-amarillenta, luminosidad intermedia, baja saturación y tonalidades con matices rojizos. Las diferencias cromáticas solo serían perceptibles, en una evaluación sensorial, entre la bebida con 25% v/v de *cold brew* y aquellas con 65 y 90% v/v de *cold brew*, así como el *cold brew* no fermentado. Los resultados evidencian la viabilidad tecnológica del uso de *cold brew* como sustrato para la fermentación de kombucha, constituyendo una alternativa potencial al té verde tradicionalmente utilizado en su producción.

Palabras clave: Bebida fermentada, Cultivo simbiótico, Cold brew, Caracterización.

ABSTRACT

Kombucha, a beverage obtained through the symbiotic fermentation of tea, has gained increasing prominence and consumer acceptance due to its functional properties and probiotic potential. In the pursuit of innovation in aroma and flavor profiles, coffee represents a promising substrate for kombucha production, while the cold brew extraction method has attracted attention because of its refreshing characteristics, reduced bitterness, and distinctive sensory attributes. Therefore, this study aimed to develop and physicochemically characterize a fermented 100% Arabica cold brew beverage produced using a kombucha symbiotic culture. Beverages containing different proportions of cold brew and green tea (25, 45, 65, and 90% v/v) were formulated, and the fermentation process was monitored through analyses of total soluble solids, pH, and total titratable acidity at 9, 15, 23, and 30 days of fermentation. Physicochemical characterization included the determination of reducing sugars, phenolic compounds, and color parameters. During fermentation, increases in total soluble solids and total titratable acidity, accompanied by a reduction in pH values, were observed, with final values remaining within the acceptable limits established by Normative Instruction No. 41/2019. The reducing sugar content of fermented samples ranged from 21.49 to 37.39 g/100 mL. The beverage formulated with 90% cold brew exhibited the highest values of pH, acidity, and reducing sugars. All fermented beverages showed a significant reduction ($p < 0.05$) in total polyphenol content compared with non-fermented cold brew. Conversely, the concentrations of other phenolic compounds increased after fermentation. Among the formulations with higher cold brew concentrations, flavonoid, flavonol, and total anthocyanin contents were higher than those observed in beverages containing greater proportions of green tea. Total tannin content was significantly higher ($p < 0.05$) only in beverages containing 45% v/v cold brew (41.31 mg/100 mL) and 90% cold brew (59.77 mg/100 mL) compared with non-fermented cold brew (31.92 mg/100 mL). Regarding color parameters, fermented beverages exhibited reddish-yellow coloration, intermediate luminosity, low saturation, and hue angles with reddish nuances. Chromatic differences would only be perceptible in a sensory evaluation

between the beverage containing 25% v/v cold brew and those containing 65 and 90% v/v cold brew, as well as the non-fermented cold brew. These findings demonstrate the technological feasibility of using cold brew as a substrate for kombucha fermentation, representing a potential alternative to the green tea traditionally employed in kombucha production.

Keywords: Fermented beverage, Symbiotic culture, Cold brew, Characterization.

INTRODUCTION

In recent years, there has been a growing demand for functional foods and beverages that are minimally processed, free from chemical additives, and characterized by high nutritional value (Maia *et al.*, 2020). According to a survey conducted by Tetra Pak, consumption of functional products by Brazilian consumers increased by 58% following the COVID-19 pandemic (ABIA, 2022). This phenomenon reflects a shift in consumer behavior regarding food choices, with increasing preference for products associated with scientifically proven health benefits (Suhre; Hickert, 2023).

Fermented beverages, such as kombucha, have recently gained popularity in countries including the United States, Canada, Australia, and Brazil due to their functional properties (Bishop *et al.*, 2022). According to Polaris Market Research (2025), kombucha market trends show its consolidation as a health-oriented beverage sector, with the global market estimated at USD 5.51 billion in 2025 and predicted to increase at a 7,50% compound annual growth rate (CAGR) until 2034. Furthermore, market growth and expansion are expected, mainly driven by increasing consumer demand, growing interest from health-conscious consumers, international market investments, and the expansion of the healthy beverage sector. Nevertheless, the commercialization of kombucha still faces important challenges related to ensuring product safety and quality (de Oliveira *et al.*, 2023).

Kombucha is traditionally produced from *Camellia sinensis* tea with the addition of sugar and subsequently fermented by a symbiotic culture of bacteria and yeast (SCOBY – Symbiotic Culture of Bacteria and Yeast) (Bishop *et al.*, 2022; Suhre, Hickert, 2023). According to the Identity and Quality Standard (PIQ) for kombucha, regulated by Normative Instruction N° 41 of September 17, 2019, issued by the Brazilian Ministry of Agriculture and Livestock, kombucha is defined exclusively as a beverage obtained from the infusion or extract of *Camellia sinensis*, either pure or combined with other herbs (Brasil, 2019).

Nevertheless, several studies have investigated the use of alternative substrates as raw materials for kombucha production, demonstrating their suitability for the fermentation process (Miranda, 2021; Zavala, 2021; Vicente Filho, 2023; Rodrigues; Matos; Furtunato, 2019).

Coffee is one of the most widely consumed beverages worldwide, and its consumption is

associated not only with its sensory attributes but also with its recognized health-promoting potential, since it constitutes an important source of antioxidant compounds associated with a reduced risk of chronic diseases, cardiovascular disorders, neurodegenerative diseases, and cancer (Muñoz *et al.*, 2020; Chavez; Caetano; Mendonza, 2022; Alcântara, 2019). Due to its chemical composition rich in bioactive compounds, coffee presents significant potential for the development of novel beverages, including kombucha-based products (Miranda, 2021; Vicente Filho, 2023).

Cold brew is a cold coffee extraction method that may be obtained through either drip or immersion processes (Barroso, 2021). This extraction method produces beverages with sensory characteristics distinct from those obtained through conventional hot extraction methods, resulting in greater smoothness, reduced bitterness and acidity, and enhanced sweetness (Silva *et al.*, 2022). According to several authors, cold extraction also promotes differences in the concentration of compounds such as chlorogenic acids (Barroso, 2021), caffeine, and phenolic compounds (Yust; Wilkinson; Rao, 2023).

Therefore, further studies addressing the characterization and chemical behavior of fermented Arabica cold brew beverages are required, considering the limited number of studies investigating the fermentation of cold brew coffee using kombucha symbiotic culture. Moreover, Brazil is the world's largest coffee producer, which may contribute to the economic feasibility of this beverage formulation (Vicente Filho, 2023).

In this context, the present study aimed to develop and perform the physicochemical characterization of 100% Arabica cold brew fermented with a symbiotic kombucha culture. Considering the growing consumption of functional and nutraceutical foods, as well as the increasing scientific interest in the physicochemical composition of kombucha, this study is justified by the importance of proposing alternative substrates for kombucha production, thus contributing to the diversification of fermented beverages.

THEORETICAL BACKGROUND

According to Normative Instruction N° 41 of September 17, 2019, kombucha is defined as “a fermented beverage obtained through aerobic respiration and anaerobic fermentation of wort produced from the infusion or extract of *Camellia sinensis* and sugars by a microbiologically active symbiotic culture of bacteria and yeast (SCOBY)” (Brasil, 2019).

Although kombucha has only recently gained popularity in Western societies, its origin is considered ancient and remains uncertain. It is presumed to have originated in the Manchuria region of China around 220 b.C., receiving the name “kombucha” only in 414 a.C., after allegedly being introduced to Japan by a physician named Kombu to treat digestive disorders of Emperor Inkyo

(Vicente Filho, 2023; Maia *et al.*, 2020). From the 1960s onward, kombucha became increasingly popular in several European countries due to studies conducted by Swedish researchers that associated its benefits with those attributed to yogurt consumption (Miranda, 2021; Maia *et al.*, 2020). In Brazil, the first mention of kombucha dates back to 1887 (Santos, 2021).

Currently, kombucha is classified as a functional beverage and has attracted increasing interest due to its reported beneficial properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, and probiotic activities, in addition to potential effects on blood pressure and cholesterol reduction (Etgeton, Janette, 2020; Maia *et al.*, 2020; Bishop *et al.*, 2020; Vicente Filho, 2023; Ferreira, 2022).

With the publication of Normative Instruction N° 41/2019, Brazil became the first country to establish specific legislation for kombucha. Internationally, the National Environmental Health Association (NEHA) published, in 2013, guidelines regarding risk analysis and fermentation procedures for kombucha under the FDA (Food and Drug Administration) Food Code Model. These guidelines allow the use of alternative substrates for kombucha fermentation, provided that the initial pH does not exceed the original tea pH (~5) (Miranda *et al.*, 2022; Miranda, 2021).

Similarly, the Kombucha Brewers International (KBI) established a code of practice aimed at ensuring safety and quality standards for kombucha production. This document includes definitions related to ingredients, finished products, hygienic requirements, processing conditions, labeling, and analytical standards. According to the KBI guidelines, substrates such as coffee, yerba mate, herbs, and sugar substitutes such as honey may be used alongside or instead of *Camellia sinensis*. Furthermore, the beverage pH should remain between 2.3 and 3.8, since acidity is considered one of the most important parameters during fermentation due to its association with the antimicrobial and functional properties of kombucha (Kombucha Brewers International, 2021; Miranda *et al.*, 2022).

The symbiotic culture of bacteria and yeast, known as SCOBY, consists of a gelatinous cellulosic biofilm responsible for the fermentation process and microbial stability of the beverage. This culture acts as the inoculum during kombucha production, generating a new SCOBY layer during each fermentation cycle (Moreno, Stiebe, 2021; Santos, 2021). Kombucha fermentation is characterized by the occurrence of acetic, lactic, and alcoholic fermentations due to the microbial diversity present within the culture (Augustinho *et al.*, 2022; Maia *et al.*, 2020). However, the metabolic activity and microbial interactions occurring throughout kombucha fermentation have not yet been fully elucidated, highlighting the need for further investigations into the dynamics of this microbial consortium.

Among the alternative substrates proposed for kombucha production, coffee has gained attention due to its sensory characteristics and chemical composition rich in bioactive compounds. In

this context, cold brew is a cold coffee extraction method that can be performed either by immersion or cold dripping. In the immersion method, ground coffee remains in contact with water under refrigeration or at room temperature for extended periods ranging from 6 to 24 hours, followed by filtration. In the cold drip method, cold water slowly passes through ground coffee retained in a filter, and the beverage is collected at the end of the process (Silva *et al.*, 2022; Barroso, 2021).

Consequently, cold brew has become an important trend in the coffee market, particularly within the ready-to-drink beverage segment, whose market value was projected to reach approximately USD 26 billion by the end of 2025 (Girardi, 2023). Compared to conventional hot extraction methods, cold brew beverages exhibit distinct sensory properties, including lower bitterness and acidity, smoother flavor, and enhanced sweetness (Silva *et al.*, 2022). Moreover, previous studies have demonstrated that cold extraction may alter the concentration of compounds such as chlorogenic acids, caffeine, and phenolic compounds (Angeloni *et al.*, 2019 apud Barroso, 2021; Pan *et al.*, 2023 apud Yust, Wilkinson, Rao, 2023; Yust, Wilkinson, Rao, 2023).

Therefore, the use of cold brew as a substrate for kombucha fermentation may result in beverages with distinct physicochemical and sensory characteristics, representing a promising alternative for innovation in fermented beverage production.

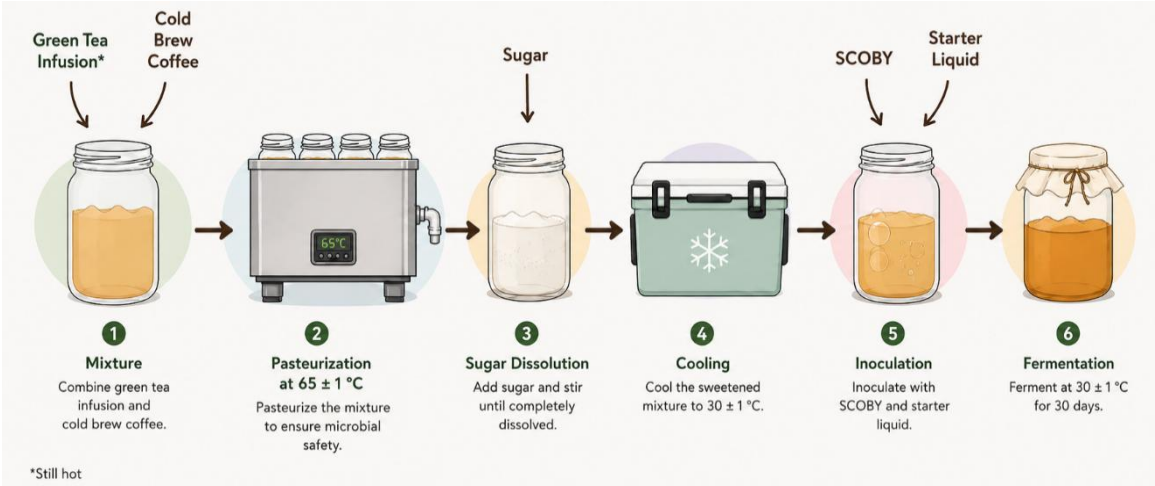
METHODOLOGY

Specialty ground coffee (100% Arabica, *Catuai Vermelho* variety) produced in the district of Miracica, Garanhuns, Pernambuco, Brazil, was used for the experiments. Cold brew coffee was prepared by cold solid–liquid extraction through immersion using 4 g of ground coffee per 100 mL of water at $22^{\circ} \pm 1^{\circ}\text{C}$ for 2 h, followed by filtration using a Koar® filter support according to conventional filtration procedures (ABIC, 2021; Agnoletti *et al.*, 2019).

Green tea infusion was prepared according to Antolak, Piechota and Kucharska (2021) using 5 g/L of green tea leaves infused in water at $85^{\circ} \pm 1^{\circ}\text{C}$ for 10 min, followed by filtration.

Fermented beverages were prepared by mixing different proportions of cold brew and green tea in previously sterilized glass containers. Crystal sugar was added at 20% m/v, followed by homogenization for 5 min. After cooling the mixtures to $30^{\circ} \pm 1^{\circ}\text{C}$, kombucha symbiotic culture (SCOBY) and 10% v/v starter liquid from a previous green tea kombucha fermentation were inoculated (Figura 1) (Rodrigues, Lopes, Coelho, 2021; Miranda, 2021 adapted). Fermentation was carried out at $30^{\circ} \pm 1^{\circ}\text{C}$ under aerobic conditions.

Figura 1: Preparation steps of cold brew beverage fermented with a kombucha symbiotic culture (SCOBY).



Source: Author's own elaboration (2024).

Four formulations containing different proportions of cold brew and green tea were prepared, with the final volume adjusted to 300 mL for each beverage. The formulation compositions are presented in Table 1. Fermented beverages were identified as KC1–KC4 according to their respective formulations, while the non-fermented cold brew sample was designated as CB. Prior to inoculation, all formulations were pasteurized at $65^\circ \pm 1^\circ\text{C}$ for 2 min and 52 s to minimize the risk of microbial contamination.

Tabela 1: Formulation composition of fermented cold brew beverages, including cold brew, green tea, sugar, starter liquid, and SCOBY concentrations used in the scaled-up production process.

Samples	Green Tea (mL)	Cold brew (mL)	Sugar (g)	Starter (mL)	SCOBY (g)
KC 1	135	135	60	30	~11,52
KC 2	75	195	60	30	~11,52
KC 3	195	75	60	30	~11,52
KC 4	-	270	60	30	~11,52

Source: Author's own elaboration (2024).

Fermentation monitoring was performed through physicochemical analyses of total soluble solids (TSS), pH, and total titratable acidity (TTA). TSS and pH measurements were conducted in triplicate at 9, 15, 23, and 30 days of fermentation, while TTA was evaluated at 15, 23, and 30 days.

Total soluble solids were determined using a manual refractometer and expressed as °Brix. pH values were measured using a digital pH meter calibrated with standard buffer solutions (pH 4.0

and 7.0). Total titratable acidity, expressed as acetic acid percentage, was determined by titration with 0.1 M NaOH using phenolphthalein as an indicator (Instituto Adolfo Lutz, 2005).

The obtained data were organized and analyzed using descriptive line graphs generated in Excel for Windows.

All spectrophotometric analyses were performed after 30 days of fermentation. Reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) method, according to Miranda (2021), with modifications, absorbance reading at 540 nm, and results expressed in g/100 mL of glucose. Phenolic compound analyses were performed in both fermented, and non-fermented beverages to evaluate the effect of fermentation on the phenolic composition.

Total polyphenols were determined using the Folin–Ciocalteu method at 725 nm, and results were expressed as mg.L^{-1} gallic acid equivalents (GAE) based on a calibration curve ($y=0,03112x+0,028$, $r^2=0,991$) (Giovannelli and Buratti, 2009).

Total flavonoids were determined according to Kim *et al.* (2003) using sodium nitrite, aluminum chloride, and sodium hydroxide reagents, with absorbance measured at 510 nm, and results were expressed as mg.L^{-1} catechin equivalents, based on a calibration curve ($y=0,03112x+0,028$, $r^2=0,991$).

Total tannins were quantified using the vanillin-HCl method according to Perez *et al.* (1999) with modifications. Absorbance was measured at 500 nm, and results were expressed as mg.L^{-1} catechin equivalents based on a calibration curve ($y=0,0013x+0,0075$, $r^2=0,999$).

Total anthocyanins and flavonols were determined according to Lees and Francis (1972), with absorbance measured at 535 nm and 374 nm, respectively. Results were expressed as mg/L cyanidin-3-glucoside equivalents for anthocyanins and mg.L^{-1} quercetin equivalents for flavonols.

Color analyses were performed on both fermented beverages (after 30 days of fermentation) and unfermented cold brew samples, using the CIELAB color system. Color parameters L^* (luminosity), a^* (red-green component), and b^* (yellow-blue component) were measured using a CR-400 colorimeter (Konica Minolta). Chroma (C^*) and hue angle (H^*) were calculated according to the equations proposed by Caillé *et al.* (2010) and Harbertson and Spayd (2006). Total color difference (ΔE) between the samples was calculated according to Monagas *et al.* (2006).

All analyses were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) was applied to identify significant differences among samples using Excel for Windows®.

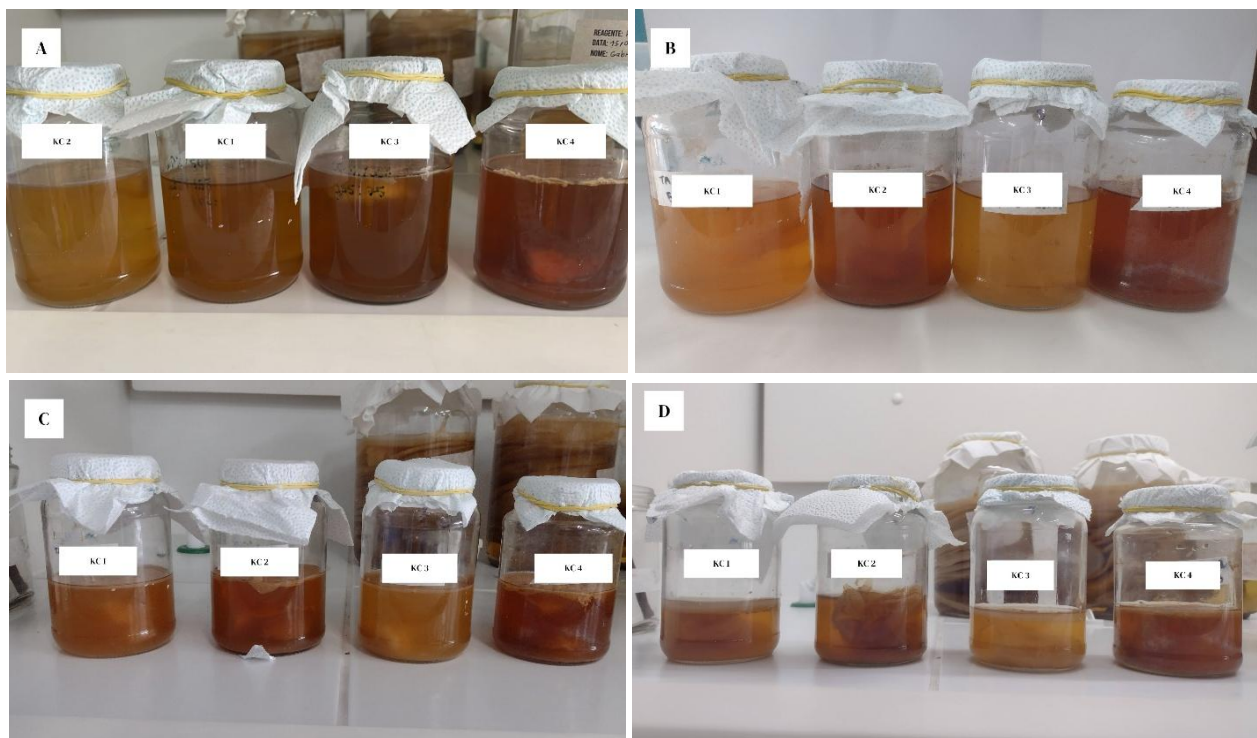
RESULTS AND DISCUSSION

During fermentation, progressive visual and olfactory changes were observed, indicating the

development of the fermentative process (Figure 2). In the first days, the beverages presented initial biofilm formation and relative clarity (Figure 2A). By the 9th day, all samples exhibited complete SCOBY formation, increased turbidity, and a slight acidic aroma (Figure 2B), which may be associated with microbial growth and cellulose production by acetic acid bacteria (Dartora, 2023).

At 15 days, thicker biofilms and characteristic kombucha aromas, including acidic, sweet, yeasty, and vinegary notes, became more evident (Figure 2C). According to Jayabalan et al. (2014), after approximately 14 days of fermentation, a new SCOBY layer is typically formed over the liquid surface while the original inoculum remains submerged.

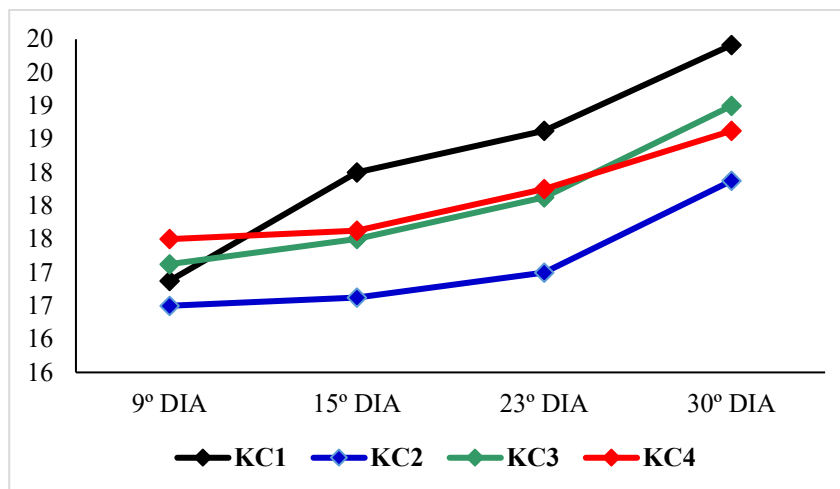
Figure 2: Changes in the appearance of cold brew kombucha formulations during fermentation (A) Day 7: initial cellulose pellicle formation; (B) Day 9: complete SCOBY formation and increased turbidity; (C) Day 15: development of thicker biofilms and characteristic fermentation features; (D) Day 30: final stage of fermentation, showing differences in color and SCOBY morphology among formulations.



Source: Author's own elaboration (2024).

Between 15 and 30 days, a substantial increase in biofilm thickness was observed, particularly in samples with higher cold brew concentrations (Figure 2D), which also retained more pronounced coffee and fruity aromas. Similar results were reported by Cunha, Santos and Schneider (2021), who observed that coffee-based kombucha showed cellulose biofilm yields comparable to those obtained in green tea fermentations.

Figure 3: Evolution of total soluble solids in cold brew coffee kombucha formulations during 30 days of fermentation.

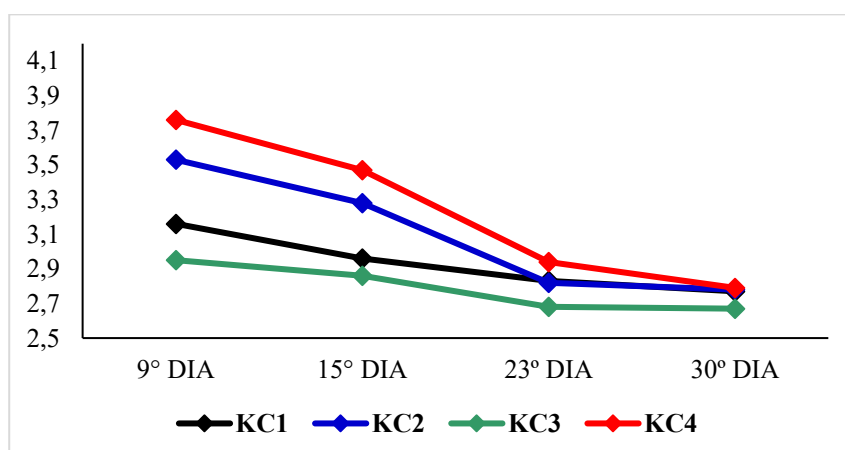


KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew.

Source: Authors' own elaboration (2024).

TSS values gradually increased throughout fermentation in all formulations, ranging from 16.8 to 19.9 °Brix (Figure 3). This behavior may be associated with sucrose hydrolysis promoted by bacterial and yeast enzymes during fermentation (Vicente Filho, 2023; Coelho *et al.*, 2020). These results differ from those reported for green tea kombucha, in which TSS decreased during fermentation due to sugar consumption by microorganisms (Rodrigues, Lopes and Coelho, 2021; Leal, 2022).

Figure 4: pH evolution of cold brew coffee kombucha formulations during 30 days of fermentation



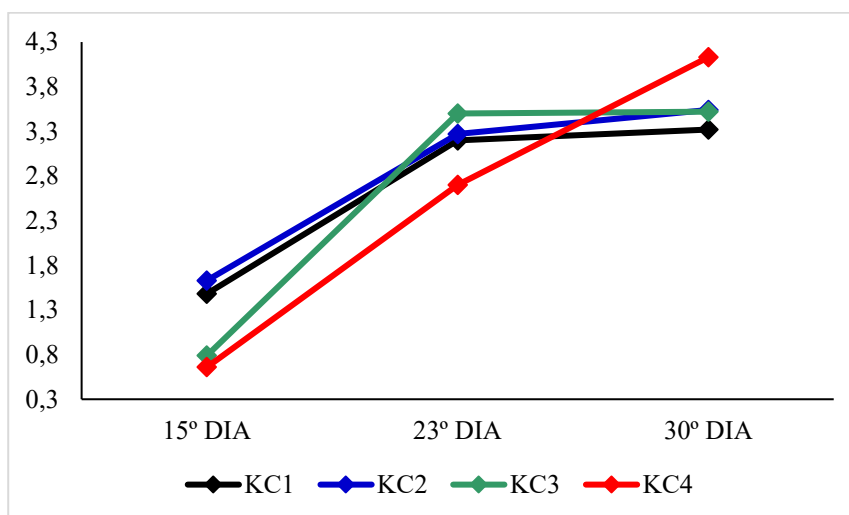
KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew.

Source: Author's own elaboration (2024).

In contrast, pH values progressively decreased during fermentation in all treatments, reaching final values between 2.67 and 2.79 after 30 days. Simultaneously, total titratable acidity increased

throughout fermentation, particularly in sample KC4, which reached the highest acetic acid concentration (3.30 mg/mL) (Figure 5). According to the *Kombucha Brewers International* (KBI), the final acidity values remained within the recommended range for kombucha beverages.

Figure 05: Evolution of titratable acidity, expressed as acetic acid, in cold brew coffee kombucha formulations during 30 days of fermentation



KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew.

Source: Author's own elaboration (2024).

The reduction in pH and increase in acidity are characteristic of kombucha fermentation and are associated with microbial metabolism, in which sucrose is converted into glucose and fructose and subsequently metabolized into ethanol and organic acids, mainly acetic and glucuronic acids (Jayabalan *et al.*, 2014). Similar trends have been reported in previous studies involving kombucha fermentation (Etgeton and Janette, 2020; Leal, 2022; Miranda, 2021). Furthermore, all formulations remained within the safe pH range established by Brazilian legislation for kombucha beverages (pH 2.5–4.2) (Brasil, 2019).

Reducing sugar contents ranged from 21.49 to 37.39 g/100 mL, as seen in Table 2, with KC4 presenting the highest concentration. Samples KC1 and KC2 showed the lowest values and did not differ significantly from each other ($p < 0.05$).

Table 2: Reducing sugar content of fermented coffee beverages (KC).

Sample	Reducing sugars (g of glucose/100ml)
KC1	21,49 ^c ± 0,69

KC2	22,72 ^c ± 0,84
KC3	30,39 ^a ± 0,19
KC4	37,39 ^b ± 1,35

KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

Source: Authors' own elaboration (2024).

The formation of reducing sugars is associated with the hydrolysis of non-reducing sugars by yeasts and acetic acid bacteria during fermentation (Santos *et al.*, 2019). Similar increases in reducing sugars during kombucha fermentation were reported by Souza (2022) and Perioto *et al.* (2022), while other studies observed reductions associated with microbial sugar consumption throughout fermentation (Etgeton and Janette, 2020; Freitas, 2022). According to Souza (2022), these variations are strongly influenced by fermentation time and substrate composition.

The results for total flavonoids, total polyphenols, total tannins, total flavonols, and total anthocyanins are presented in Table 3. The formulation containing the highest concentration of cold brew (KC4) showed significantly higher values ($p < 0.05$) for total flavonoids, tannins, flavonols, and anthocyanins when compared with the other fermented beverages. In contrast, total polyphenol content decreased significantly ($p < 0.05$) in all fermented samples relative to the non-fermented cold brew (CB), although KC4 still presented the highest value among the fermented formulations.

Table 3: Phenolic compound contents of fermented and non-fermented coffee beverages.

Sample	Total Polyphenols (mg/100mL)	Total Flavonoids (mg/100mL)	Total Tannins (mg/100mL)	Total Flavonols (mg/100mL)	Total Anthocyanins (mg/100mL)
CB	144.40 ± 5.19 ^a	49.31 ± 0.29 ^b	31.92 ± 0.26 ^c	4.43 ± 0.43 ^b	0.18 ± 0.02 ^c
KC1	99.89 ± 0.00 ^d	25.44 ± 1.06 ^d	41.31 ± 2.31 ^b	3.03 ± 0.56 ^{bc}	0.45 ± 0.12 ^c
KC2	106.16 ± 0.10 ^c	35.72 ± 0.19 ^c	40.69 ± 6.28 ^c	3.82 ± 0.18 ^b	0.85 ± 0.12 ^b
KC3	106.20 ± 0.05 ^c	18.32 ± 0.42 ^e	40.15 ± 1.15 ^c	1.88 ± 0.26 ^c	0.22 ± 0.05 ^c
KC4	134.04 ± 0.21 ^b	54.73 ± 0.93 ^a	59.77 ± 2.77 ^a	8.54 ± 0.06 ^a	2.89 ± 0.13 ^a

CB: non-fermented cold brew; KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

Source: Authors' own elaboration (2024)

These findings differ from several studies reported in the literature, in which fermentation promoted an increase in total polyphenols due to enzymatic biotransformation of phenolic compounds during kombucha fermentation (Bishop *et al.*, 2022; Leal, 2022; Vicente Filho, 2023). Similar increases in phenolic content were also reported in fermented Arabica coffee kombucha (Miranda, 2021) and green coffee kombucha (Vicente Filho, 2023). However, Freitas (2022) highlighted that prolonged fermentation may also reduce phenolic compounds due to polymerization reactions mediated by microbial enzymes.

The formulations with higher proportions of green tea (KC1 and KC3) showed lower concentrations of total flavonoids and anthocyanins, whereas KC4 exhibited the highest flavonol content. Total tannins increased after fermentation, with significant differences ($p < 0.05$) observed mainly for KC1 and KC4. Similar trends relating higher substrate concentration to increased phenolic compounds were reported in kombucha produced with yerba mate (Sola, 2023) and coffee substrates (Karyantina, Surulloh, Suhartaik, 2024). Nevertheless, contradictory results regarding tannin behavior after fermentation have also been described (Muzaifa *et al.*, 2023).

Variations in phenolic composition may be associated with factors such as substrate type and concentration, fermentation temperature, sugar content, inoculum characteristics, and processing conditions (Miranda, 2021). Additionally, thermal processing may influence phenolic stability, since pasteurization has been associated with reductions in polyphenol and flavonoid content in fruit beverages (Saikia *et al.*, 2016; Tobolková *et al.*, 2023).

Color analysis performed using the CIELab system demonstrated significant differences among the fermented beverages and the non-fermented cold brew (Table 4). Fermentation significantly reduced luminosity (L^*) in all treatments, particularly in KC4, indicating darker beverages. The fermented samples also exhibited increased red coloration (a^*), especially in formulations with higher cold brew concentrations, while the yellow coordinate (b^*) decreased in KC1 and KC3, suggesting reduced yellow intensity associated with higher green tea content.

Table 4: CIELab color parameters of non-fermented cold brew (CB) and fermented cold brew kombucha beverages (KC).

Sample	L^*	a^*	b^*	C^*	H°
CB	45.55 ± 1.34^a	-0.97 ± 0.03^d	17.46 ± 0.62^a	17.49 ± 0.62^b	42.06 ± 1.20^a
KC1	40.18 ± 0.40^b	0.86 ± 0.06^c	15.98 ± 0.17^b	16.00 ± 0.20^c	36.85 ± 0.36^b
KC2	36.13 ± 0.77^c	2.42 ± 0.15^b	17.35 ± 0.20^a	17.51 ± 0.18^{ab}	31.60 ± 0.78^c

KC3	37.37 ± 0.62^c	1.08 ± 0.12^c	13.97 ± 0.09^c	14.00 ± 0.08^d	34.65 ± 0.64^d
KC4	33.89 ± 0.57^d	4.08 ± 0.09^a	17.91 ± 0.38^a	18.37 ± 0.27^a	28.49 ± 0.52^c

CB: non-fermented cold brew; KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew. L: lightness; a: red–green chromatic coordinate; b: yellow–blue chromatic coordinate; C (Chroma): color intensity/saturation; H° (Hue angle): color hue expressed in degrees. Values are expressed as mean $\pm$ standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

Source: Authors' own elaboration (2024).

The fermented beverages presented low chroma values, indicating low color saturation, and hue angle values below 90°, demonstrating predominance of reddish-yellow tones. Similar reductions in luminosity and hue angle after fermentation have been reported for hibiscus kombucha (Rodrigues *et al.*, 2018). Changes in color parameters during fermentation are associated with pigment biotransformation, polymerization, and depolymerization reactions promoted by pH reduction and microbial enzymatic activity (Freitas, 2022).

Table 5: Color difference (ΔE) between non-fermented cold brew and fermented cold brew kombucha formulations

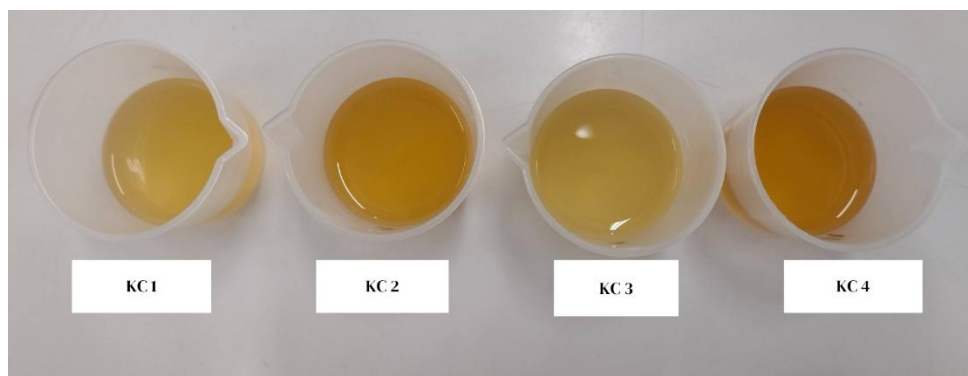
Amostras	ΔE
KC1 e KC2	1,51
KC1 e KC3	2,00
KC1 e KC4	2,37
KC1 e CB	1,48
KC2 e KC3	3,51
KC2 e KC4	0,85
KC2 e CB	0,03
KC3 e KC4	4,36
KC3 e CB	3,48
KC4 e CB	0,88

CB: non-fermented cold brew; KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew.

Source: Authors' own elaboration (2024)

The chromatic difference (ΔE) analysis demonstrated perceptible color variation only between KC3 and the samples KC2, KC4, and CB, with ΔE values above 3, which according to Monagas et al. (2006) represents a difference perceptible to the human eye (Table 5; Figure 6). Overall, the fermented beverages were characterized by reddish-yellow coloration, medium luminosity, and low color saturation.

Figure 6: Fermented beverages produced with different proportions of cold brew and green tea (v/v) using a kombucha symbiotic culture after 30 days of fermentation



CB: non-fermented cold brew; KC1: 45% (v/v) cold brew and 45% (v/v) green tea; KC2: 65% (v/v) cold brew and 25% (v/v) green tea; KC3: 25% (v/v) cold brew and 65% (v/v) green tea; KC4: 90% (v/v) cold brew.

Source: Authors' own elaboration (2024)

Although kombucha is an ancient beverage, it has attracted increasing attention from researchers due to the growing consumer interest in nutraceutical and functional foods. From a commercial perspective, the kombucha market is projected to experience substantial growth in the coming years, reflecting the increasing demand for health-oriented beverages and the expansion of the functional food sector (Polaris Market Research, 2025; Miranda *et al.*, 2022). Furthermore, the growing diversification of kombucha products highlights its potential for market expansion and innovation (Barros, Botelho, Chisté, 2024).

In addition to its nutritional and sustainability-related benefits, one of the main attractions of kombucha is its distinctive sensory profile. Consequently, considerable research efforts have focused on modifying fermentation substrates, applying post-fermentation flavoring strategies, and enhancing carbonation to diversify the beverage and improve consumer acceptance (Wang *et al.*, 2022; Barros, Botelho, Chisté, 2024; Villarreal-Soto *et al.*, 2022).

In this context, the present study demonstrated the feasibility of producing fermented kombucha beverages using 100% Arabica cold brew as substrate. The results contribute to the

understanding of physicochemical changes occurring during cold brew fermentation and may support future studies involving sensory acceptance, antioxidant activity, and the use of SCOBY cultures adapted specifically to coffee substrates.

CONCLUSIONS

The production of fermented cold brew using a kombucha symbiotic culture under the proposed processing conditions proved to be feasible, as evidenced by the physicochemical changes observed during fermentation, particularly the reduction in pH and the increase in acetic acid concentration, confirming microbial metabolic activity. Moreover, the final pH values remained within the limits established by Brazilian Normative Instruction No. 41, ensuring beverage safety.

Total soluble solids and reducing sugar contents remained elevated after fermentation, likely due to the conversion of sucrose into glucose and fructose by yeasts, followed by the metabolism of glucose into fructose and gluconic acid by acetic acid bacteria.

The phenolic composition varied according to the proportions of green tea and cold brew used in the formulations. Higher cold brew concentrations promoted greater increases in phenolic compounds after fermentation, whereas formulations containing higher green tea concentrations showed the opposite behavior. These findings suggest that cold brews represent a promising substrate for kombucha production.

Regarding color parameters, fermentation altered the beverage color from greenish yellow in the non-fermented cold brew to reddish yellow in the fermented beverages. The green tea-to-cold brew ratio directly influenced the CIELab color coordinates. Samples with higher cold brew concentrations were darker, with lower hue angle values, higher color saturation, and greater red and yellow intensity. In contrast, formulations containing higher proportions of green tea resulted in lighter beverages with higher hue values, lower saturation, and reduced a^* and b^* coordinates.

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