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Coffee-Flavoured Kombucha: Development, Physicochemical Characterisation, and Sensory Analysis

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Abstract: Considering the health benefits of kombucha, already widely studied, the objective of this study was to develop kombucha beverages with the addition of an infusion of specialty arabica coffee from the first fermentation, varying the proportion of specialty coffee (2% to 13%), and evaluate their pH, volatile acidity, degree of alcohol, centesimal composition, sodium contents, and colour parameters to determine the acceptability of the beverages. Concerning the pH, all of the formulations conformed with the kombucha identity standard, but K3 (11% coffee) and K4 (13% coffee) were below the established minimum for volatile acidity. Except for K4, all of the other formulations were classified as alcoholic kombuchas, although their values were very close to the limit for non-alcoholic beverages. The formulations presented low sugar and sodium contents, which corroborated their low caloric value. Therefore, coffee-flavoured kombucha fermented for 18 days becomes an option for consumers looking for low-calorie, refreshing, and healthy drinks. The luminosity of the beverages decreased as the proportion of coffee increased, and consequently, the values for a* and b* increased, indicating a strong tendency for a yellowish-red colour. Sensorially, the formulations K4 and K3 were the most well accepted in all respects and can therefore be considered formulations with high commercialisation potential.

Keywords: process; innovation; arabica coffee; *Camellia sinensis*; acceptability

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1. Introduction

Consciousness of the role of diet in promoting health is constantly increasing, and hence people are searching for foods and beverages with allegations of health properties [1–3]. Thus, functional beverages such as kombucha fit the perspectives of different consumers as functional foods and can be considered healthy drinks [4].

Various studies have shown that the consumption of kombucha is associated with antioxidant [5–7], antimicrobial [8,9], and antiproliferative [6,10] properties; a reduction in triglyceride levels; the treatment of digestive diseases; and stimulation of the immunological system [11–14]. The beverage also contains metabolites such as organic acids, vitamins, minerals, and phenolic compounds [15,16].

Kombucha, which originated in the region of Manchuria (China), is a mixture of sugar with tea fermented by a symbiotic culture of bacteria and yeasts (*SCOBY*) [17]. Black or green teas are the most used teas and sucrose is the most used sugar, but currently, other substrates with bioactive properties are being studied and used, such that the beverage presents functional properties [18,19]. To be considered as a functional food, it must conform with the following requisites: it must be a food product, have scientific evidence sustaining the benefit of the product, show measurable physiological effects, and be consumed daily as part of a normal diet [20].

Of the microorganisms capable of carrying out these conversions, *Acetobacter xylinum* is the bacterium most mentioned, plus the yeast genera *Brettanomyces*, *Zygosaccharomyces*,

Saccharomyces, and *Pichia* [21,22]. According to Leal et al. [23], kombuchas are a source of bioactive components such as polyphenols and glucuronic acid. Lactic acid bacteria transform the kombucha's glucose into gluconic acid and fructose into acetic acid, while the yeast hydrolyses the sucrose, producing fructose and glucose. This true symbiosis between the yeast and the bacteria produces a cellulose pellicle known as a *SCOBY*, which remains suspended in the tea, containing the metabolites [24].

The presence and amounts of metabolites depend on the types of microorganisms in the symbiotic culture used to ferment the kombucha, as well as the duration of time, the temperature, the amount of sucrose, and the type of tea used in the fermentation process [25,26]. The fermentation time is an important factor, influencing the antioxidant and sensory properties of the fermented product, which suffers a sudden fall in pH value and a consequent increase in acidity, a characteristic typical of this beverage [27,28].

Sensorially, kombucha can be described as a refreshing, slightly sweet, carbonated, and acidic functional tea, which helps promote health by way of a symbiotic culture containing acetic acid bacteria and yeasts [23]. Yuliana et al. [29] used cocoa honey as the fermentation substrate for the tea in the presence of *SCOBY*, and observed that the acidity and total soluble solids decreased after six days of fermentation, confirming the good sensory quality of their kombucha beverage. Sales et al. [30] developed coffee-leaf kombucha and their beverage presented natural and potentially healthy aromatic compounds and a satisfactory sensory acceptance.

The beverage kombucha can present a natural flavour or a typical flavour caused by using either diverse fruits such as apples [31], cocoa [29], coffee [28], or diverse flowers, amongst other ingredients such as the milk used in the initial culture [32].

In addition to being an extremely popular beverage, coffee contains bioactive compounds, as do *Camellia sinensis* teas, to which health benefits are attributed, with the principal components being caffeine and phenolic compounds—catechins in tea and chlorogenic acids in coffee [33–35]. Coffee is one of the most popular and consumed drinks in the world, and Brazil is the country that leads its production, with 66.4 million bags being produced in 2024 [36]. This market tends to continuously grow due to consumer habits and satisfaction, as well as the diversity of varieties—and, consequently, flavours—available, in addition to innovations with raw materials. Amongst the coffee classifications, we can find traditional coffee and specialty coffee. Specialty coffee indicates coffee with a score above 80 points on a scale between 0 and 100 points, awarded according to a sensory evaluation made by a Q Grader (a professional taster specialised in specialty coffees) using the Specialty Coffee Association (SCA) methodology [37].

The objective of this study was to elaborate a kombucha with the addition of specialty coffee from the first fermentation and evaluate the physicochemical, colorimetric, and sensory characteristics of the beverage. A simple study design was used, where the coffee infusion was the only variable and the percentage of *Camellia sinensis* green tea was fixed. The beverages were analysed after 18 days of fermentation (5 days of aerobic fermentation and 13 days of anaerobic fermentation).

2. Materials and Methods

2.1. Materials

The green tea used was from the Leão brand (Brazil).

The specialty coffee used was 100% arabica Red Catuai (IAC H 2077), originating in Natércia (MG, Brazil) and distributed by Coffee Run Bike, considering the sensory evaluation described on the label.

The fermentation process took place in 3 L glass jars. The glass jars used were sterilised at 121 °C for 20 min. During the fermentation process, the glass jars were covered with paper towels and secured with elastic tape. The production setup was kept in the dark, at an average temperature of 28.8 °C.

2.2. Elaborating Process

The flowsheet in Figure 1 shows the process used for the coffee-flavoured kombucha beverages up to their 1st fermentation, with the description of each part of the flowsheet described in steps.

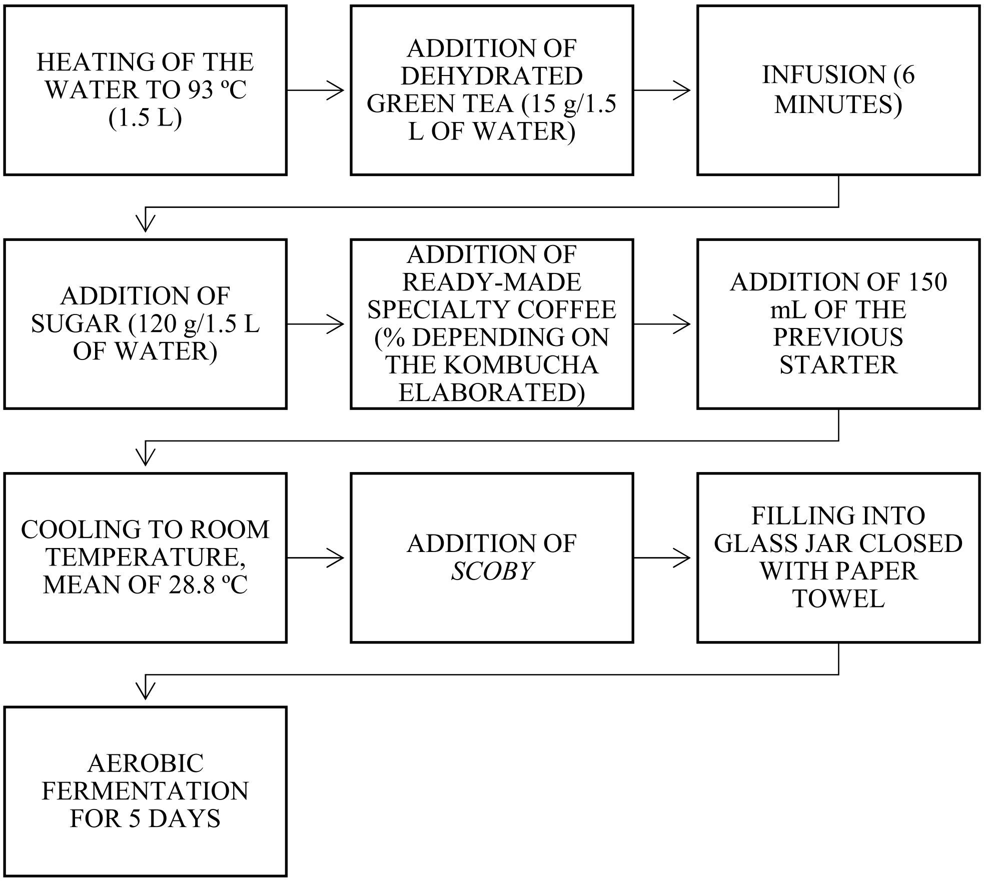

Figure 1. Flowsheet of the first fermentation of the kombuchas.

2.2.1. First Step: Preparation of the Coffee Infusion

The coffee infusion was produced using V60 equipment (Hario, Tokyo, Japan). A quantity of 10 g of coffee powder was placed in contact with 100 mL of water (hot-brew process/extraction at 93 °C with the V60 filtration equipment). This filtration method allowed for efficient extraction of the compounds, conferring a mild sweetish flavour on the beverages and thus producing the coffee infusion used to elaborate the kombucha beverages. Due to the filter's single large hole and the grooves at an angle of 60° from the base to the top of the filter, the air managed to escape without impeding the expansion of the coffee grounds caused by the vapour.

2.2.2. Second Step: Obtaining the *SCOBY* Cultures and Preparing the Materials

Two *scobys*, weighing between 200 g and 300 g, were donated by a kombucha micro-company located in the city of Salvador (Bahia, Brazil). Each *scoby* was added to a 3 L glass jar containing 200 mL of tea, 200 g of sugar, 150 mL of starter (tea from the previous kombucha), and 1500 mL boiled mineral water. The formation of 4 new *scobys* (biofilms) was observed in each jar, which was divided between 4 more glass jars, giving a total of 6 jars, each containing *scobys* of equivalent weight, with the same individual conditions described previously (Figure 2). This whole process was carried out under controlled conditions regarding the temperature and moisture content, which were maintained at about 28 °C and 69%, respectively.

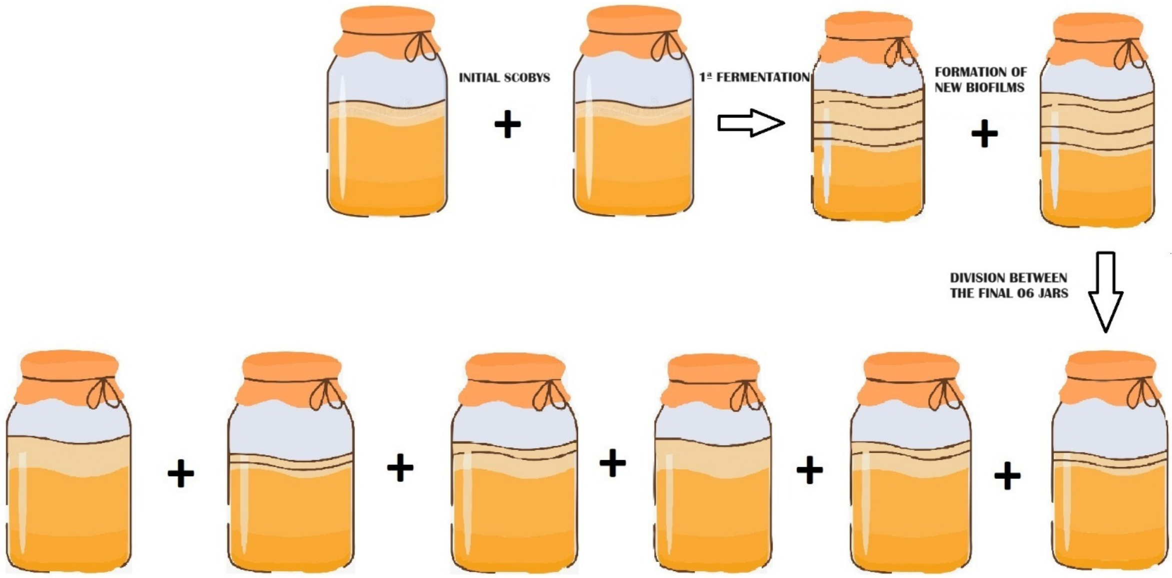

Figure 2. Fermentation of initial *scobys* for new biofilm formation.

2.2.3. Third Step: Experimental Design

Various preliminary tests were carried out with different fermentation times and amounts of ingredients until a kombucha beverage with an acceptable flavour was obtained. A sensory evaluation was carried out with kombucha-beverage consumers with a daily consumption frequency, using a 3-point scale (disliked; neither liked nor disliked; liked). A kombucha denominated as the control was obtained and used to elaborate the subsequent formulations with different concentrations of infused coffee (Table 1).

Table 1. Experimental design for the coffee-flavoured kombucha formulations.

Formulation	*Scoby* (g)	Water (mL)	Ready-Made Green Tea (mL)	Infused Specialty Coffee (mL)	Starter (mL)	Sugar (g)
K1	±250	1500	160	40	150	120
K2	±250	1500	120	80	150	120
K3	±250	1500	80	120	150	120
K4	±250	1500	40	160	150	120

2.2.4. Preparation of Kombucha Beverage Formulations with Different Coffee Proportions

The biofilm samples (*scobys*) did not have identical weights since the materials were of a natural origin, but they all contained, on average, between 200 g and 300 g.

After the first derivation (when the original *scoby* created another biofilm), tests were carried out with the previously mentioned proportions of 13% of green tea and 2% of coffee; 11% of tea and 4% of coffee; 4% of tea and 11% of coffee; and 2% of tea and 13% of coffee (Figure 2).

After 5 days of aerobic fermentation, the beverages were bottled, 7 g of sugar was added, and the bottles were sealed and left for 13 days at room temperature. Anaerobic fermentation occurred during this step of the process, with the formation of gas. The liquid was then transferred to 370 mL glass bottles, refrigerated for 2 days at 7 °C, and then analysed (Figure 3).

Figure 3. Bottled kombucha formulations (K1—2% coffee; K2—4% coffee; K3—11% coffee; K4—13% coffee).

2.3. Sensory Analysis

Before the sensory analysis, the formulations were analysed for their microbiological innocuity, for the safety of the evaluators.

A preliminary sensory analysis was carried out during the fermentation processes to adjust the fermentation time according to the flavour agreeability, on a scale of disliked, neither liked nor disliked, and liked.

One hundred and twenty acidic-beverage consumers from the academic community and invited external kombucha appreciators took part in the sensory acceptance test for the four formulations denominated as K1, K2, K3, and K4. The participants evaluated the acceptance of each beverage's colour, aroma, and flavour using a 10-point scale with extremes of 'disliked extremely' and 'liked extremely', intermediated by the term 'neither liked nor disliked' [38]. The samples, refrigerated at 7 °C, were coded with three digits, balanced using a complete block design, and presented uniformly with drinking water at room temperature to rinse the palate.

The test was applied in the Sensory Analysis Laboratory of the Faculty of Pharmacy (UFBA) according to the ABNT NBR ISO 13299 norms. The project was appreciated and approved by the Ethics in Research Committee of the Faculty of Pharmacy/UFBA under report number CAAE: 57013122.4.0000.8035.

2.4. Physicochemical Analyses

Some parameters, such as the pH value, alcoholic degree, and volatile acidity, were analysed according to the methodologies described in NI (Normative Instruction) n° 41/2019 [39].

The pH was determined using a KASVI (K38-1465) pH-meter.

To determine the alcoholic degree of each sample, 100 mL of the sample was placed in the distillation flask of an Electronic Hydrostatic Balance, mod. Superalcomat (Gilbertini, Italy), together with 20 mL of water. A volume of 80 mL was collected and the alcoholic °G was read using the Oenochemical Electronic Distilling Unit Super D.E.E. (Gilbertini, Italy). The volatile acidity was also determined using the Oenochemical Electronic Distilling Unit Super D.E.E. (Gilbertini, Italy) with 20 mL of the sample. The reducing and total sugar contents, and the ash, moisture, lipid, and protein contents, were determined according to the AOAC methodologies [40] indicated for beverages. Total sodium was analysed using atomic emission spectrophotometry (AES) (Varian Spectra AA 55B, Mulgrave, Victoria, Australia).

A volume of 275 mL of each kombucha formulation was used to determine these parameters.

2.5. Colorimetric Analysis

The colorimetric parameters were determined using a Konica Minolta colorimeter (Chroma Meter CR-5) in the transmittance mode with a standard D65 luminant and a 10° observational field, according to the standardization of the *Commission Internationale de l'Eclairage* (CIELabe CIEL*C*h) system and the OIV norms (2022) [41]. A 10 mm thick 5 mL glass cuvette was used to measure the colour of the samples in triplicate, determining the values for luminosity (L* = 0 or L* = 100), red/green colour component (a+* and a−*), blue/yellow colour component (b+* and b−*), chroma (C*), and hue (h). The colour tests were carried out in triplicate.

2.6. Microbiological Analyses

The four samples were tested for the presence of *Salmonella* and *Escherichia coli*, and negative results were obtained for these bacteria, thus confirming the safety of the kombuchas according to the parameters of Guide 23 of 2019 [42].

2.7. Statistical Analyses

The data were analysed statistically using the XLSTAT program to carry out a Univariate Variance Analysis (ANOVA) and Tukey's test, considering $p = 0.05$.

3. Results

Table 2 shows the physicochemical analysis results for the kombucha formulations. Amongst the four formulations, the total sugar contents showed no significant differences. The sugar content is an important parameter since it can be associated with the sensory acceptance of the product, apart from it being an important indicator of the nutritional formation of the beverage. The sugar contents among the formulations ranged from 5.8 to 7.0 g/100 g; when compared with the sugar values usually present in soft drinks, our formulations (K1 to K4) contain 32 to 40% less sugar. Soft drinks, generally, have a sugar content between 8.5 and 11.8 g/100 g [43].

Table 2. Physicochemical data describing the coffee kombucha formulations, with the standard deviations.

Parameter	Formulation			
	K1	K2	K3	K4
pH	2.86 ± 0.13^{b}	3.08 ± 0.04^{a}	3.11 ± 0.01^{a}	3.22 ± 0.01^{a}
Volatile acidity, g/L	3.23 ± 0.06^{a}	2.17 ± 0.18^{b}	1.73 ± 0.04^{c}	1.02 ± 0.01^{d}
Alcoholic degree, °G	0.93 ± 0.06^{a}	1.23 ± 0.06^{b}	0.67 ± 0.04^{c}	0.28 ± 0.03^{d}
Total sugar, g/100 mL	5.85 ± 0.28^{a}	5.84 ± 0.43^{a}	6.42 ± 1.01^{a}	7.00 ± 0.86^{a}
Moisture, g/100 mL	93.64 ± 0.22^{a}	93.72 ± 0.12^{a}	93.26 ± 0.28^{a}	92.53 ± 0.14^{b}
Ash, g/100 mL	0.04 ± 0.00^{a}	0.06 ± 0.03^{a}	0.05 ± 0.06^{a}	0.07 ± 0.01^{a}
Lipid, g/100 mL	0.66 ± 0.26^{b}	0.78 ± 0.07^{b}	1.45 ± 0.36^{a}	1.48 ± 0.13^{a}
Protein, g/100 mL	0.10 ± 0.00^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.02^{a}
Sodium, mg/100 mL	0.61 ± 0.01^{b}	0.70 ± 0.02^{a}	0.70 ± 0.04^{a}	0.75 ± 0.04^{a}
Caloric value, kcal/100 mL	35.09	37.39	38.70	38.96

Means in the same line followed by the same superscript lowercase letter indicate no significant difference ($p > 0.05$).

The ash and protein contents exhibited no significant differences between the four formulations. Concerning the pH value, all of the formulations presented values that characterized the formulations as kombuchas, but about their volatile acidity, only that of K4 meets the legislation standard. The attributes of the total sugar, ash, and protein contents resulted in no significant differences between the four samples. However, there was a significant difference of more than 5% for the pH value, the volatile acidity, the alcoholic degree, and the sodium, moisture, and lipid contents.

Considering their caloric value, the formulations showed values between 35.09 and 38.96 kcal; in caloric terms, our formulations are similar to standard soft drinks, which are usually 39 to 42 kcal [43]. These formulations (K1, K2, K3, and K4) can be substitutes for soft drinks, with the advantage of having low sugar concentrations.

Regarding sodium, the concentration varied from 0.61 to 0.75 mg/100 mL in our formulations. This range of values is considered low when compared to that of standard soft drinks, which have an average sodium concentration of 8 to 11 mg/100 mL [43].

Table 3 shows the values obtained for the colour parameters, and it is possible to observe excellent discrimination between the formulations according to the colour parameters. Notably, the higher the coffee concentration, the higher the values for C* (colour purity), a*, and b*, suggesting K4 as a beverage tending towards a more reddish-brown colour compared to the others. The a* values vary according to the increase in coffee concentration, which went from −0.8 to 1.9 within 18 days of fermentation. Miranda et al. [28] found values of a* = 0.62 using a 2% coffee infusion in their preparation of kombuchas.

Table 3. Means obtained for the colour parameters of the coffee kombucha formulations.

Formulation	L	a*	b*	C*	h°
K1 (2% coffee)	97.5 ^{a}	−0.8 ^{d}	17.1 ^{d}	17.1 ^{d}	92.7 ^{a}
K2 (4% coffee)	93.1 ^{b}	−0.2 ^{c}	25.3 ^{c}	24.4 ^{c}	90.6 ^{b}
K3 (10% coffee)	90.3 ^{c}	0.1 ^{b}	28.7 ^{b}	28.7 ^{b}	89.7 ^{c}
K4 (13% coffee)	86.9 ^{d}	1.9 ^{a}	36.4 ^{a}	36.5 ^{a}	86.8 ^{d}

Means in the same column followed by the same superscript lowercase letter indicate no significant difference ($p > 0.05$). Abbreviations: L = luminosity; a* = +red/−green coordinate; b* = −yellow/+ blue coordinate; C* = chroma; h° = hue.

Concerning the sensory analysis, the evaluators carried out an acceptance test for the attributes of colour, aroma, and flavour (Figure 4). Before carrying out the sensory analysis, the four formulations (K1, K2, K3, and K4) were tested for *Salmonella* and *Escherichia coli*; by obtaining negative results for these pathogenic bacteria, that is, results conforming to the parameters in Brazilian legislation [42], we confirmed the safety of our kombuchas as adequate for consumption.

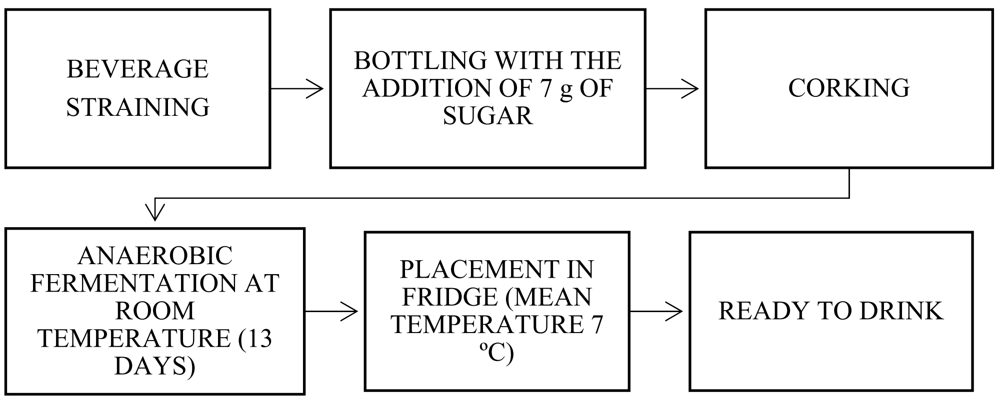

Figure 4. Flowsheet of the second fermentation of the kombuchas.

The variance analysis (ANOVA) ($p = 0.05$) of the evaluation data for the kombucha samples showed no significant differences between the formulations for the colour and aroma data. However, there was a significant difference between the samples to flavour, with K3 and K4 receiving the highest scores (Table 4). It is important to consider that the acceptability scores for the flavour of these two formulations were greater than six: that is, these scores were further away from the zone of doubt or rejection on the 10-point scale.

Table 4. Means obtained for the sensory attributes of the coffee kombucha formulations.

Formulation	Colour	Aroma	Flavour
K1 (2% coffee)	6.1 [a]	5.0 [a]	5.4 [b]
K2 (4% coffee)	6.0 [a]	5.1 [a]	5.7 [b]
K3 (10% coffee)	5.8 [a]	5.3 [a]	6.0 [ab]
K4 (13% coffee)	6.3 [a]	5.7 [a]	6.9 [a]

Means in the same column followed by the same superscript lowercase letter indicate no significant difference ($p > 0.05$).

4. Discussion

According to Brazilian legislation, to be considered kombucha, a beverage should present values of volatile acidity, alcoholic degree, and pH within specific determined ranges to guarantee its identity standard. Of the proposed formulations, only kombucha K4 was not classified as an alcoholic kombucha. There was probably a slowdown in its fermentation, as coincidentally K4 also had the highest concentration of sugar, indicating that the sugar was not consumed in fermentation.

To the pH value, all of the formulations presented values that characterized them as kombuchas. The pH value is one of the most important environmental parameters that affects the fermentation of kombucha, since some of the acids formed, such as acetic and gluconic acids, can be responsible for the biological activities of the resulting beverages [44].

The formulation K4 showed the highest indices for the total sugars, the pH value, and the ash and lipid contents, together with K3, and presented the highest sodium index.

For the volatile acidity, only K1 and K2 qualified as kombucha, and concerning the alcohol content, only K4 could be considered non-alcoholic, with a value of <0.5% v/v. Regarding the pH value, all of the formulations were within the established parameters of from 2.5 to 4.2 [39]. The coffee concentration probably influenced the development of fermentation by the yeasts or bacteria, since a slightly higher pH value was observed upon increasing the coffee concentration when compared with the beverage prepared with the smallest coffee concentration. The same behaviour was observed for the volatile acidity: that is, the acidity decreased upon increasing the coffee concentration.

It is important to consider that fermentation in kombucha is carried out by yeasts and bacteria, and each class presents different behaviours depending on the chemical composition and humidity. This possible inhibition can be explained by the residual total sugar concentrations of formulations K4 and K3 at the end of fermentation (Table 2). Sales et al. [45] found similar values for the pH of kombucha with an 80% coffee infusion and a fermentation time of 9 days.

The sodium levels, an important and mandatory parameter of technical regulation RDC n° 429/2020 [46], ranged from 0.61 to 0.75 mg/100 g. RDC n° 429/2020 [46] describes the standards for declaring the nutritional information of food products, which require information on the amount of sodium in the product to be provided on the product's label. When comparing the sodium contents of our formulations (K1 to K4) with the levels of this element in standard soft drinks, it can be seen that our kombuchas have around 91% less sodium than standard soft drinks. Having a high sodium intake is linked to having high blood pressure, which compromises health and increases the risk of cerebrovascular accidents (CVAs) and myocardial infarction.

Comparing the caloric value of our kombucha formulations with soft drinks, the difference is very small, but kombucha has bioactive compounds that bring benefits to the health of the gastrointestinal system. The possibility of replacing soft-drink consumption with kombucha consumption is very beneficial for your health, as the amount of common sugar and preservatives ingested will be lower.

The colour of kombucha drinks is an important parameter, as it is slightly cloudy and slightly yellowish. Adding coffee to the preparation of kombucha causes significant changes in colour parameters [28]. Thus, the values of the a* parameter indicate a redder drink when increasing the coffee concentration. The variation in *a (−0.8 to 1.9) is due to the increase in the concentration of coffee used in preparation; these values are proportional to those found in ref. [28], but the colour depends on the coffee used and its preparation. For the parameter of colour, kombucha K4 was the most appreciated for the parameter of color, with a mean score of 6.35; this was followed by K1, with 6.10; K2, with 6.03; and K3, with 5.84. Formulation K4 presented the following values for the colour parameters—a* (1.9), b* (34.5), C* (36.5), and L (86.9); hence, the highest acceptance for the colour of the kombuchas was associated with the highest concentrations of coffee.

Concerning flavour, the most appreciated kombucha was again K4, with a mean of 6.96, followed by K3 with 6.07. We might suggest that the colour of the kombuchas might have influenced the acceptance of the flavour concerning the darker beverages. Using functional magnetic resonance imaging (fMRI), Osterbauer et al. [47] showed the influence of colour on flavour and odour. These authors described the perception of colour–odour congruency. Therefore, these colour–flavour interactions are probably real.

The mean acceptance scores for K1, K2, K3, and K4 ranged from 5 to 6.9. Recently, research has found acceptance scores for kombucha formulations with added coffee-leaf tea of between 5.4 and 6.6 using a nine-point hedonic scale [30]. Our results show that kombucha K4, with the highest coffee percentage, was the most accepted amongst the evaluators for all parameters. This was also the sweetest of the beverages, with the lowest acidity, and the only one considered non-alcoholic, which might have influenced the evaluators' perception. According to Rossini and Bogsan [48], before NI n° 41/2019 [39], there was no control of kombucha production in Brazil, and in 2021, almost 100% of the kombuchas produced were classified as alcoholic, a result not favourable to the proposal of kombuchas.

On the other hand, K1 was the least accepted of the kombuchas for the parameters of aroma and flavour, this being the kombucha closest to the original kombucha beverage, resulting in a more acidic beverage with a lower sugar content and the highest alcoholic degree (Table 2). This formulation showed the highest volatile acidity, the smallest colour purity value (C*), the and highest luminosity (L).

We suggest that the use of coffee in the production of kombucha presents a positive impact on the acceptance of these beverages. We also suggest refining the production process and carrying out new analyses, such as those assessing antioxidant activity and antimicrobial effects, since this study focused on the development and procurement of a coffee-flavoured kombucha beverage. It is also important to control the production process to obtain non-alcoholic kombucha to increase the impact on its consumption.

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Informed Consent Statement: Written informed consent has been obtained from the consumers to publish this paper.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

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