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Cocoa Mucilage: A Novel Substrate for Fermented Tea-Based Beverages

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ABSTRACT

Cocoa mucilage, predominantly comprised of sugars, yeasts, and minerals, poses a potential wealth in fermentation processes. This study explores its under-exploited potential by investigating its use as a sugar substitute in the production of a fermented beverage, thereby adding value to what is often dismissed as waste and extending its applications. The research was designed to assess the effects of varying concentrations of cocoa mucilage in the fermentation process of the Symbiotic Culture of Bacteria and Yeast (SCOBY), with a focus on producing a beverage based on green and black tea. The experimental design incorporated factors such as the concentration of mucilage (15, 20, and 30%) and the type of tea (green or black). The process of producing the fermented beverage was meticulously described, followed by comprehensive physicochemical (including pH, brix, alcohol, acidity, among others), microbiological (E. coli, Salmonella, yeasts, and bacteria), and sensory (colour, odour, flavour, sweetness, and astringency) analyses. Six experimental units were formulated by modulating the mucilage concentration and the tea type. The outcomes demonstrated pH values of 3.58, 2.90 °Brix, 0.136% acidity, a density of 0.98, turbidity of 10.4 NTU, 1.61 °GL, and the absence of any microbiological contamination. The combination of 20% mucilage concentration with black tea (a1b1) received the highest approval in the sensory analysis, with an average score of 7.09/10.00 from 18 testers. In industrial applications, cocoa mucilage could be harnessed as a fermentative source due to the presence of *Saccharomyces Servisiae* yeast type, which facilitates sugar oxidation in fermented beverages. Thus, this research proposes an alternative use for cocoa mucilage, contributing to waste reduction and broadening its potential applications.

1. INTRODUCTION

Ecuador is recognised as a leading exporter of cocoa to European and North American markets, attributed to the superior aroma and flavour of the product, which results in a consistently high demand [1, 2]. The cocoa plant, belonging to the dicotyledonous class, family Malvaceae, and genus Theobroma, thrives at altitudes of 0-1200 meters above sea level (m.a.s.l.), influencing the fruit characteristics and genetic material variability [3, 4].

Over time, each species accumulates genetic information within its population, which is then transmitted to subsequent generations [5]. Despite possessing shared characteristics, individual variants may abound within a species' population, leading to genetic variability that enables adaption to environmental changes [6].

Cocoa fruit mucilage, collected from two sources - the cob's shell and tree bark, and the cocoa beans and the maguey - exhibits divergent properties. The former type has a flocculant capacity, useful for clarifying sugar cane juice, while the latter is utilised in the production of distilled and non-distilled liquors [7].

In countries such as Peru, the benefits of cocoa liquid or mucilage are harnessed in the production of products like liquor, marmalade, and jellies. However, in Ecuador, the use of this residue is limited due to inadequate government support and industry knowledge regarding mucilage benefits, leading to the unnecessary discarding of this valuable by-product [8, 9].

Cocoa mucilage, a viscous substance contained within cocoa plants, possesses remarkable physicochemical characteristics including sugars, vitamins, and minerals [10, 11]. These attributes confer sensory properties such as a pleasant flavour and aroma. Countries such as Brazil, Costa Rica, and Colombia utilise mucilage in food manufacturing, though confusion persists between mucilage and pectins, which differ only in their physical properties [12, 13].

Kombucha, a fermented tea beverage, is renowned for its bioactive tea compounds and essential components such as acetic acid, D-saccharic acid-1,4-lactone, glucuronic, and gluconic acids [14, 15]. These compounds, produced via the metabolic activities of bacteria and yeasts, confer health benefits [16]. Kombucha contains a symbiotic culture of bacteria and yeast (SCOBY), responsible for fermenting the sugar [14].

The fermentation of sweetened tea at room temperature over several days using a cellulose film comprised of acetic acid bacteria and yeast results in Kombucha [17]. The beneficial

health effects of Kombucha have led to the inclusion of various traditional plants in its preparation [14]. SCOBY, a by-product of Kombucha tea fermentation, is currently being explored for its potential use as a raw material in diverse fields such as food technology, biomaterial preparation, the textile industry, and environmental biotechnology [10].

The slightly acidic nature of Kombucha arises from the fermentation of sweetened black tea by the Kombucha microbial consortium, resulting in a beverage high in antioxidants [11]. The chemical composition of Kombucha varies depending on factors such as fermentation time, tea substrates, and the microorganisms present in the inoculum. However, components like organic acids, vitamins, polyphenols, and amino acids are typically present [12].

Arbutus Kombucha, with its remarkable nutritional and aromatic properties, could serve as an alternative beverage, with fermentation time and SCOBY composition identified as key factors [15]. The Kombucha fungus, formed by a consortium of yeasts and bacteria, is used to produce a fermented beverage with hypoglycemic, anti-inflammatory, antihypertensive, and antioxidant properties. This beverage is obtained by fermenting black or green tea with sugar [18].

Different countries are studying the microbial composition of the Kombucha fungus or SCOBY. Several studies report that this consortium comprises acetic bacteria, including *Acetobacter xylinum, Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti* and *Acetobacter pasteurianus* and *Gluconobacter*. Yeasts such as *Schizosaccharomyces pombe* and *Saccharomycodes ludwigii* have also been identified [19, 20]. Several industrial sectors benefit from SCOBY, such as the food industry, biotechnological processes, and biomedicine [21-23].

However, its production rarely occurs on an industrial scale, and studies evaluating its fermentation process are scarce. The use of Kombucha and SCOBY to produce beverages has been increasing, such as fermented milk beverages [24, 25], red grape juice [26] or soymilk [27]. Furthermore, different sweeteners (e.g., sugar cane and coconut sugar) are used for various types of homemade Kombucha beverages [28].

Consequently, this study aims to evaluate the application of cocoa mucilage as a sugar source in the SCOBY fermentation process to produce a fermented beverage. Six combinations of Kombucha beverages sweetened with varying proportions of cocoa mucilage and cane sugar were prepared. The beverage with the highest cocoa mucilage to cane sugar ratio was noted to have the best sensory properties, including flavor, aroma, and color. The results of this study suggest that cocoa mucilage could be a viable alternative to traditional sweeteners in the production of Kombucha beverages.

In conclusion, cocoa mucilage has potential as a sugar source in the SCOBY fermentation process for the production of Kombucha beverages. More research is required to understand the optimal conditions for fermentation and the potential health benefits of these beverages. This could lead to the development of new, innovative products in the beverage industry and a reduction in waste from cocoa production.

2. MATERIALS AND METHODS

The bromatological analyses conducted for this research on the fermented beverage were performed in the laboratory facilities of the State Technical University of Quevedo (UTEQ), as shown in Figure 1.

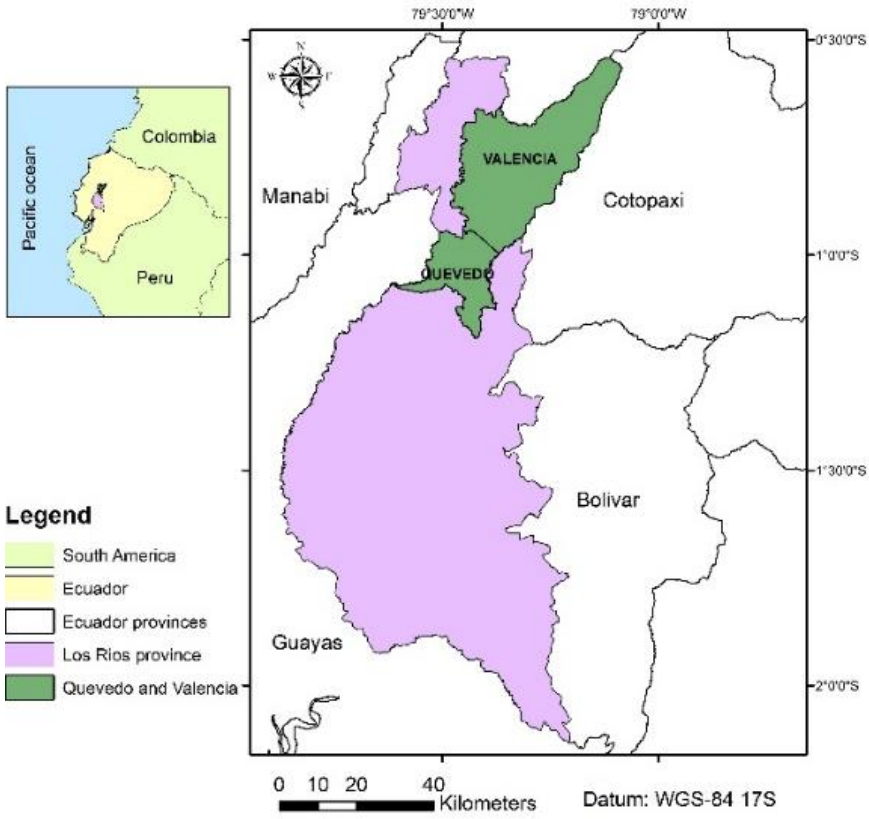

Figure 1. Location of the study area and raw material sourcing sites

The sub-products or raw materials used to make the fermented beverage were green tea, black tea, water, cocoa mucilage, and SCOBY. Cocoa mucilage, SCOBY and tea were obtained in Valencia (Los Rios province).

SCOBY is a symbiotic colony of yeasts and bacteria that produce different beverages and foods (e.g., kefir, Kombucha, Mother of Vinegar, Ginger Beer Plant) [20]. SCOBY has a role in the fermentation process of Kombucha and changes its physical and chemical characteristics. After fermentation, the Kombucha liquid is produced, which is acidic and high in chemical content [29]. As Kombucha contains a variety of microorganisms, active ingredients and uses a fermentation process, it has a wide range of healthcare functions (e.g., antioxidant, liver protection, cardiovascular, cerebrovascular, and anticancer) [30].

The methodology used in this research consisted of applying an experimental method (physicochemical, microbiological, and sensory analyses). Also, the inductive-deductive method was used to identify the current problems and provide a solution with the appropriate technology.

2.1 Design of research combinations

A factorial block design (A×B) model was used in the present investigation. Factor A was the cocoa mucilage concentrations (15, 20 and 30%), while factor B was the two types of tea used for the fermented beverage production process (green tea and black tea) (Table 1).

Table 1. Factorial block design (A×B)

Number	Symbology	Description
1	a0b0	15% mucilage + Green tea
2	a0b1	15% mucilage + Black tea
3	a1b0	20% mucilage + Green tea
4	a1b1	20% mucilage + Black tea
5	a2b0	30% mucilage + Green tea
6	a2b1	30% mucilage + Black tea

Tukey's test was used to determine the effects between conditions and treatments ($p<0.05$) and the handling of statistical data. Tukey's test compares the individual means by analysing the variance of several samples subjected to different treatments [31].

2.2 Description of the fermented beverage production process

The process of elaborating the fermented cocoa mucilage beverage is described in the following steps:

(1) *Conditioning of materials:* The container prepared can be metallic (such as a pot) or glass (bottles or containers) and must be sterilised to start packaging.
(2) *Tea pasteurisation:* Tea is pasteurised with one litre of water in this operation. Two or three tea bags were used until the colour looked a little dark, helping to prevent light from filtering through and interrupting the fermentation process. Pasteurisation is performed at 100°C for 5 minutes, resulting in a concentrated solution.
(3) *Tea cooling*: This operation allowed the water and tea solution to stand until its temperature decreased to approximately 25°C for the following mixing.
(4) *Mucilage pasteurisation:* The cocoa mucilage was at 80°C for about 5 minutes. The temperature is not raised further because it could affect the composition or structure of the mucilage.
(5) *Cooling of mucilage:* In this operation, the mucilage is left to rest and cool until it descends to 25°C, below which mixing is possible.
(6) *Homogenised:* This operation serves for two or more solutions to be mixed. Once the tea and mucilage reach 25°C, the two solutions are placed in the same container. Mixing is done very slowly for correct integration. Here the percentage of cocoa mucilage is applied to the mixture as appropriate (15, 20 or 30%).
(7) *Fermentation:* The liquid is then poured into the glass container containing the SCOBY until it completely covers the SCOBY. Then, it is well sealed with filter paper or absorbent towels for the correct intervention of oxygen and gases emitted during the process. It should be stored dry, away from light and heat. It is left for seven days.
(8) *Sieving:* In this operation, attention is given to avoiding small lumps visible after fermentation caused by the yeast and cocoa mucilage.
(9) *Packaging:* After seven days, the product is packed. It must be filtered and then packaged in bottles (previously disinfected).
(10) *Stored:* Storage is carried out under refrigeration to decrease the temperature and stop the fermentation process (or slow it down significantly).

2.3 Physicochemical parameters analysis

For the physicochemical analyses, the following tests were carried out:

(1) *Determinación del pH:* An OHAUS Starter 5000 pHmeter was used. Samples of 50 mL of each fermented beverage were placed in a beaker (NTE INEN - ISO 10523 standard [32]).
(2) *Turbidity determination:* For the turbidity determination, a HACH model 2100AN turbidity meter was used (INEN 971 standard [33]). The previously calibrated equipment measures the intensity of the scattered light at 90 degrees. Each treatment was placed in 30 mL glass buckets. Then, the samples were placed on the turbidimeter support and waited 15 minutes to observe and record the lecture expressed in NTU (Nephelometric Turbidity Unit).
(3) *Acidity determination:* For the acidity determination, we followed the procedure shown in INEN 13 [34] using the following Eq. (1):

$$\%\ \text{lactic acid} = \frac{0.1 * V_{NaOH} * 0.09}{Pm} * 100 \quad (1)$$

By titration of 10 mL of a sample with 0.1 NaOH and using phenolphthalein as an indicator as a percentage of lactic acid.

(4) *Brix determination (°Brix):* For the Brix determination, an ATAGO refractometer was used, following the procedure of the INEN 273 standard [35].
(5) *Alcohol determination (°GL):* The alcoholic levels were measured with an AL AMBIK alcoholmeter (0-100%), as indicated in the INEN 340:1994 standard [36]. For the determination of alcoholic strength, a sample with 150 mL of sample was used for each treatment.
(6) *Density determination:* The pycnometer is washed and rinsed with distilled water, following the procedure

shown in INEN 923:2013 [37]. The beverage density is determined using the following Eq. (2):

$$Dr = \frac{Mm - Mt}{Ma - Mt} \quad (2)$$

where,

Mm: Mass present in the pycnometer (g.).

Ma: Representative mass of the pycnometer with water (g.).

Mt: Empty pycnometer mass (g.).

Dr: Relative density.

The determination should be made in duplicate of the same prepared sample, as follows: a) Weigh the clean and dry pycnometer to the nearest 0.1 mg., b) empty the pycnometer, clean it, dry it internally with a stream of dry air and fill it with distilled water up to the respective mark (avoiding the formation of air bubbles).

(7) *Absorbance and transmittance determination:* The work was carried out with a spectrophotometer measuring both values simultaneously. The procedure was based on standard Mexican NMX-AA-051/1-SCFI-2008 [38]. A spectrophotometer with a wavelength of 430 nanometers was used, applying the Beer-Lambert law for absorption. The spectrophotometer, instead of intensity, measures absorbance. Using absorbance in spectra has the advantage of being directly proportional to the molecule concentration in the sample.

2.4 Microbiological parameters

For microbiological analyses, the following tests were performed:

(1) *Determination of E. coli and Salmonella:* To determine the presence of E. coli and Salmonella, sowing in Petri dishes is performed. It was left in the Memmert Incubator at 28°C for 48 hours, following the steps indicated in INEN 1529-7 [39]. E. coli was analysed with MacConkey Agar, and the result of the analysis was determined within 48 hours with the colony count according to the INEN standard [40].

(2) *Determination of yeasts and bacteria:* The procedure used for E. coli and Salmonella determines the presence of yeasts and bacteria. The respective sowing is done in Petri dishes, and then they are taken to the Memmert Incubator at 28°C for 48 hours. The procedure indicated in the INEN 1205:2013 standard is followed [41]. In each case, 1 mL of the dilution was sown in duplicate and incubated at 25°C for five days; for moulds and yeasts, 1 mL was sown in copy and set at 25°C for five days.

2.5 Sensory analysis

The characteristics of the beverage were evaluated by sensory analysis. The product tasting was conducted by 18 tasters aged 25-45 years. Each person had samples containing 20 ml of the beverage.

The products presented to the panellists for evaluation are safe to drink and harmless to health. Previously, the components used in each experiment were explained, as well as the concentrations of cocoa mucilage and the types of tea used. The purpose of the study was explained to the panellists, and whether they were interested in participating and contributing to this research.

A 10-point scale was applied for each parameter: colour, odour, flavour, sweetness, and astringency. The scale established was as follows: 2 points=rejecting, 4=undesirable, 6=satisfactory, 8=desirable, and 10=very desirable.

3. RESULTS

An experimental design was applied to the six experimental units of Table 1 (with three replicates for each experimental unit), using Tukey's test to determine the effects between levels and treatments ($p<0.05$). Tukey's test is the most widely applied and preferred test by statisticians since it controls in a better way the two widely known errors in statistics (a y β). This test allows all possible comparisons of two-by-two treatments to be made and is therefore considered the most complete.

3.1 Physicochemical parameters

Table 2 shows the means of the physicochemical analyses performed in the Experimental Units (Table 1).

Tables 2 and 3 show the difference between means established by Tukey's significance test between A×B interactions. Regarding pH values, the highest value was recorded at a0b1 (15% concentration + black tea), with a value of 3.71. In acidity, the most representative value was 0.18, found at a2b0. In acidity, the most representative value was 0.18, found in a2b0. In °Brix, a1b1 obtained a value of 3.77 (the highest recorded). For density, the highest value was 1.02 in a1b1 (20% concentration + black tea). As for °GL, the highest value was reported for a1b1 (2.07). Regarding turbidity, the highest value was at a1b0 with 15.24. Concerning absorbance, the highest value was 1.20 in a1b1 (20% concentration + black tea). Finally, in transmittance, the highest value was in a1b0 with 64.63.

Table 2. Average results obtained from the physicochemical analysis of the different experimental units

Symbology	pH	Acidity (%)	°Brix (°)	Density (g/L)	°GL (°)
a0b0	3.38	0.07	1.83	1.01	1.01
a0b1	3.71	0.09	170	0.96	0.94
a1b0	3.26	0.14	2.33	1.01	1.28
a1b1	3.71	0.09	3.77	1.02	2.07
a2b0	3.14	0.18	3.27	1.01	1.80
a2b1	3.58	0.11	2.47	1.01	1.36

Table 3. Average results obtained from the physicochemical analysis of the different experimental units (continued)

Symbology	Turbidity (NTU)	Absorbance (mg/L)	Transmittance (mg/L)
a0b0	13.66	0.60	25.87
a0b1	7.48	1.13	8.00
a1b0	15.24	0.35	64.63
a1b1	9.86	1.20	6.57
a2b0	12.27	0.65	22.87
a2b1	8.04	1.09	8.17

3.2 Microbiological results

Table 4 shows the results concerning the fermented beverage in the A×B interaction of the microbiological analyses.

Table 4. Average results obtained from the microbiological analyses of the different experimental units

Symbology	Total Coliforms		Total Microorganisms	
	Salmonella (CFU/g)	E. Coli (CFU/g)	Bacteria (CFU/g) ($\times 10^6$)	Yeast (CFU/g) ($\times 10^6$)
a0b0	< 5	< 5	0.00	0.37
a0b1	< 5	< 5	0.00	0.51
a1b0	< 5	< 5	0.00	0.64
a1b1	< 5	< 5	0.00	0.74
a2b0	< 5	< 5	0.00	1.09
a2b1	< 5	< 5	0.00	1.28

Table 4 shows the results obtained after the total coliform count, where no presence was found in any interaction, thus demonstrating that the product is in perfect condition. Concerning total microorganisms, no presence of bacteria was found. Meanwhile, in yeast, it was shown that a2b1 (black tea + 30% concentration) had the highest value of colonies (1.28×10^6). However, the lowest in the interaction was a0b0 (green tea + 15% concentration) with 0.37×10^6 colonies.

According to INEN 1529, the data presented in Table 4 must contain a maximum of 10 CFU/g in anaerobic microorganisms. Meanwhile, regarding the standard for alcoholic beverages, NTE INEN 2262 establishes that the maximum values for moulds and yeasts are 10 CFU/g.

3.3 Sensory analysis (appearance, colour, odour and taste)

For this section, the methodology described in Section 2.5 was followed. The 18 tasters (aged 25-45 years) received three samples and were presented in identical containers, coded with three-digit random numbers. Each sample was given a different number, and all samples were presented simultaneously to each panellist in random order.

The procedure to be followed was explained to them, so the tasters were not trained; where the samples were scored on a 10-point scale in four parameters (colour, odour, taste, and appearance). Table 5 presents the following sensory analysis results:

Table 5. Mean results obtained from sensory analysis of the different experimental units

Symbology	Appearance	Colour	Odour	Taste
a0b0	8.50	5.00	8.17	8.50
a0b1	6.17	6.17	3.33	5.00
a1b0	7.33	6.17	6.17	5.00
a1b1	7.00	8.17	5.00	8.17
a2b0	8.50	7.33	8.50	6.17
a2b1	7.33	8.17	6.17	8.17

(1) In appearance, the highest value was 8.5 in the combination of a0b0 and a2b0 (green tea with a mucilage concentration of 15 and 30%, respectively).
(2) Regarding colour, the most representative values are black tea at concentrations of 20% and 30% cocoa mucilage (8.17).
(3) As for the aroma, the highest value was the combination of green tea with a 30% concentration of cocoa mucilage (8.50 in a2b0).
(4) In flavour, the highest value was 8.50 in the combination a0b0 (green tea at 15% concentration of cocoa mucilage). Black tea at 15% and green tea at 20% obtained 5.00 (the lowest recorded).

4. ANALYSIS OF RESULTS

4.1 PH

From the results in Table 2, mean values were obtained at a0=3.58, a1=3.48, and a2=3.34 (at mucilage concentrations of 15, 20 and 30%, respectively). These values are similar to those obtained by Maldonado-Jibaja et al. [42], which were 3.54-3.73. Maldonado-Jibaja et al. [42] explain that animal-source foods have a high buffering capacity (helps to maintain a stable pH), while vegetable-source foods do not. In the study by Arguedas-Gamboa [43], the pH is higher (around 4.60), even though coffee is slightly more acidic, allowing the fermentation process to break down the sugars better. In the research by Dirzo et al. [44], a pH of 4.00 was obtained in the beverage, adjusting to those obtained in this research, giving a slightly acid taste.

4.2 °Brix

Regarding factor A (mucilage concentration), a value of 1.72 was obtained in a0 (15%), 2.94 in a1 (20%), and 2.90 in a2 (30%). These values were compared with those obtained by Apaza Mamani and Choque Mamani [45], which were 7.30-7.50. They emphasise that sugar content is important in fermentation because wine yeast (*Saccharomyces cerevisiae*). In the research conducted by Sánchez-Muyulem et al. [46], they obtained values of 6.80 to 8.00° due to using pure pulp fruit juices.

4.3 Acidity

As for mucilage concentrations (%), the mean values obtained in a0 were 0.072%, in a1 0.112%, and a2 0.136%. In the studies carried out by Velázquez-López et al. [47] and Hernández-Monzón et al. [48], they obtained an acidity of 0.60% (higher than those obtained in Table 2). The study conducted by Falcón-Romero et al. [49] showed variations in acidity from 0.50-0.72% due to the degree of dilution of the capuli paste and the percentage of honey must (variation in fermentation).

4.4 Density

In the results shown in Table 2, a value of 0.982 was obtained for a0, 1.015 for a1, and 1.012 for a2. These values are similar to those obtained by Rodríguez-Villacis and Hernández-Monzón [50], which was 1.024. The study by Arteaga Solórzano et al. [51] showed density values between 1.041-1.098 because when sugar is converted into alcohol, the density value decreases. These values are quite similar to those obtained in this research.

4.5 Turbidity

As for turbidity, the following results were shown in a0, a value of 10.4 NTU; in a1, 12.4 NTU; and in a2, 10.0 NTU. According to INEN 971 [15], turbidity values should be a maximum of 40 NTU, so the results are within the permissible limit. However, in the research conducted by Vásquez and Medina [52], the results were 230.5 NTU because it is a high-concentration fermented mixed beverage. The presence of pectin and turbidity agents causes a high turbidity level. The turbidity of fresh fruit juices can be decreased by pectinase treatment.

In another investigation, Ricardo et al. [53] reported data between 58-135 NTU, depending on the beer brewed with malted and unmalted quinoa. Unlike the beverage under study, this one has a lower concentration of suspended solids that directly influence the turbidity of the beverage.

4.6 Alcohol content (°GL)

The mucilage concentration percentage was obtained at a0 0.89 °GL, at a1 1.61 °GL, and a2 1.58 °GL. According to the study by Torres et al. [54], a non-alcoholic fermented beverage must be below 1%, which is only met with a0 (15% mucilage concentration). In the study conducted by Pascual Pastor et al. [55], the results ranged from 4.5-6.0% due to the addition of yeast after the seventh day of natural fermentation.

The alcoholic content is the ratio of the volume of ethyl alcohol (ethanol) contained in a hydroalcoholic mixture, measured at 20°C. The total volume of the mixture is expressed as a volume fraction (%) [56]. The alcoholic grade level in beverages depends on the fermentation or biochemical processes of the microorganisms (yeasts).

4.7 Absorbance and transmittance

The results obtained for Absorbance (Ab) and Transmittance (Tr) for factor A were as follows: in a0, a value of Ab: 0.86 and Tr: 16; in a1, Ab: 0.77 and Tt: 36; and in a2, Ab: 0.88 and Tr: 14. Regarding absorbance, Verónica [57] shows that in its best treatment, its value is 0.735, a similar value in the present investigation. While in the research conducted by Bartolo et al. [58], the initial absorbance for "white chicha de jora" at a wavelength of 680 nm was 1.833, while for "dark chicha de jora", it was 1.942.

4.8 Microbiological analysis

Concerning the microbiological tests in Table 4, no total coliforms or bacteria were found, ensuring the final product's safety and providing safety to the consumer. However, traces of yeasts were found in the samples since they are fundamental parts of the fermentation process and act as probiotics to improve the quality and effects of the fermented beverage (based on INEN 2395) [59]. The research conducted by León et al. [60] found the presence of microorganisms and different types of yeasts and bacteria. Eight strains were isolated from conventional microbiology techniques (two may have probiotic potential). According to the study conducted by López et al. [61], no microorganisms were found in their beverage. At the same time, yeasts had a minor presence (typical of fermentation).

Table 6. Growing conditions used in the research

Microorganisms	Growing Conditions	Method
Salmonella	Salmonella Shigella Agar	INEN 767 [62]
Escherichia coli.	Agar MacConkey	INEN-2667, 2013 [63]
Bacteria	Agar Nutrient	AOAC método oficial 986.33 [64]
Molds and yeasts	Potato Dextrose Agar	INEN 767 [62]

Table 6 presents the microorganisms, growing conditions, and method (standard) used in this microbiological analysis.

4.9 Sensory analysis of fermented beverage combinations

As for the sensory analysis, the results in Table 5 show that the fermented beverage combination with the best average is a2b0 (30% mucilage + green tea), even though it obtained a low value in taste. The combination with the lowest average acceptance rate was a0b1 (15% mucilage + black tea). In the study conducted by Calvario-Palma et al. [65], it was shown that the Beb2 formula (amaranth flour) obtained the best acceptance, presenting higher percentages of appreciation and flavour, as well as the highest rating on the hedonic scale value (5) in odour and texture. On the other hand, the research conducted by Marulanda et al. [66] indicated that the sample formulated with the lowest concentration of solids had the highest preference regarding aroma and sweetness but the lowest acceptance regarding acidity and texture and resemblance to commercial yoghurt.

4.10 Limitations and future research lines

Within the analysis of the results obtained in this research, the following limitations have been presented or observed:

(1) There were limited resources within the investigation, with which certain results could not be further expanded (e.g., a greater number of physicochemical or microbiological analyses).
(2) Lack of available information on the fermentation of the Kombucha drink with cocoa mucilage. This can make it difficult to conduct comparative studies or analyse the results.
(3) A constant and specific temperature is required during fermentation (approximately 25-30°C) for processing. Appropriate hygiene measures must be taken during preparation to avoid contaminating the sample with microorganisms.
(4) The fermentation process can take 7-14 days, there may be a slightly acidic flavour change and sometimes a little astringent. For proper conservation, effective pasteurisation must be carried out with a high-pressure system at low temperatures, which requires a good investment in equipment.

Therefore, it is important to keep these limitations in mind when interpreting the research results of the Kombucha-type drink with cocoa mucilage and to consider the need for larger and more comprehensive studies to understand this drink's benefits and limitations.

Likewise, possible lines of investigation for a Kombucha-type drink with cocoa mucilage were analysed and determined; as follows:

(1) Studies on the beneficial health effects of Kombucha consumption, such as antioxidant activity, improved digestion, and immune function.
(2) Research Kombucha's composition and production process to improve its flavour, texture and nutritional properties.
(3) Analysis of the microbial strains involved in Kombucha fermentation to better understand how they interact and how they can be modified to achieve desired results.
(4) Development of large-scale production techniques and studies on the long-term preservation of the Kombucha drink.

(5) Research on the use of alternative ingredients, such as different types of tea or natural sweeteners, to improve the quality and diversity of Kombucha.
(6) Exploring new markets and business opportunities for Kombucha, such as an energy drink, sports drink, skin toner, and other health and wellness-related products.
(7) To evaluate the effect of cocoa mucilage on the intestinal microbiota and gastrointestinal health of those who consume the beverage.

The lines of research for a Kombucha-type drink with cocoa mucilage can be very diverse, from substituting carbon sources for fermentation to evaluating its effect on sensory quality and consumers' health.

5. CONCLUSIONS

Six different combinations of fermented beverages were evaluated by applying cocoa mucilage in different concentrations (15, 20, and 30%) to two different types of tea (green and black). The best combination of physicochemical and microbiological aspects was obtained at 20% cocoa mucilage concentration. Meanwhile, in the sensory aspect, the best beverage was 30% mucilage with green tea.

The physicochemical analyses were within the permissible range of national and international standards, with pH values of 3.58, 2.90 °Brix, acidity of 0.136%, density of 0.98, 10.4 NTU turbidity, and 1.61 °GL. Meanwhile, no bacteria were found in the microbiological parameters. Traces of yeast were found, but these are lower than the national standard INEN 1529. This is beneficial, as it gives greater consumer satisfaction by having a natural ingredient and beneficial yeast content.

The sensory analysis conducted in this research showed that cocoa mucilage is a great substitute for traditional sugar. The best beverage was 30% mucilage with green tea (7.09/10.00 de 18 tasters). The mucilage in the cocoa production chain has a high nutritional and functional value since it contains vitamins (e.g., B complex, C, D and E) and minerals (e.g., Ca, Fe, K, Mg and Zn). Its functional properties are due to its composition's presence of antioxidant compounds. This antioxidant capacity makes mucilage a raw material of interest for developing products to combat oxidative stress.

Regarding the fermentation time of the beverage, a range of seven days allows it to increase its acidity. This allows obtaining a product with the physical and chemical characteristics of the beverage (SCOBY) by using a 20% concentration of mucilage in combination with black tea. This is due to the presence of yeasts that aid fermentation and work better in totally dark environments but not in clear solutions such as green tea (there is a behavioural type of changes in taste, texture or nutritional content).

As for the type of tea used, the one that obtained the best results in the physicochemical and microbiological aspects was black tea; while in the sensory analysis, green tea presented a higher acceptability of the product. Among the characteristics of green tea, it has antioxidant, antimicrobial, anti-inflammatory, stimulant, antioxidant, hypoglycemic, anti-obesity, anticancer, digestive, hypolipidemic, diuretic and antiviral properties. Generally, mucilage is used to prepare smoothies and sweet drinks due to its unique flavour and quick availability. Also, cocoa juices are developed entirely from this white pulp without adding sugars.

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REFERENCES

[1] Bedrán León, C.N., Becerra Bravo, G.C. (2017). Elaboración de jugo de mucílago de cacao pasteurizado. Bachelor's dissertation. Facultad de Ingeniería en Mecánica y Ciencias de la Producción, ESPOL Polytechnic University, Guayaquil, Ecuador.
[2] Arciniega-Alvarado, G.A., Espinoza-León, R.A. (2020). Optimización de una bebida a base del Mucílago del Cacao (Theobroma cacao), como aprovechamiento de uno de sus subproductos. Dominio de las Ciencias, 6(3): 310-326.
[3] Parmawati, R., Risvita, W., Hakim, L., Rahmawati, N. O., Gunawan, F.K., Ashari, F.M., Haqqi, S.S. (2023). Sustainability index of robusta coffee plantation (Case study: Wagir district smallholder coffee plantation in Malang, Indonesia). International Journal of Design & Nature and Ecodynamics, 18(2): 279-288. https://doi.org/10.18280/ijdne.180205
[4] Bastidas, J.V., Melo, W.A.B., Briones-Bitar, J. (2022). Sustainability of the cocoa industry: Cocoa waste mucilage use to produce fermented beverages. Case study in Los Ríos province. International Journal of Sustainable Development and Planning, 17(4): 1147-1152. https://doi.org/10.18280/ijsdp.170412
[5] Syahza, A., Bakce, D., Irianti, M., Asmit, B., Nasrul, B. (2021). Development of superior plantation commodities based on sustainable development. International Journal of Sustainable Development and Planning, 16(4): 683-692. https://doi.org/10.18280/ijsdp.160408
[6] Hernández, M.D.P.L., Aldana, A.P.S., Lozano, J.G., Nuñez, J.C. (2021). Estudio morfoagronómico de materiales de cacao (Theobroma cacao L.) de diferentes zonas productoras en Colombia. Ciencia y Agricultura, 18(3): 98-109. https://doi.org/10.19053/01228420.v18.n3.2021.12570
[7] Escobar, Á.O.F., Solórzano, C.Y.E., Segarra, C.V.T., Navarrete, E.D.T., García, D.A.T., Ocampo, R.G.D. (2022). Extracción de mucílago de cáscara de Theobroma cacao L. para uso en clarificación de jugos de Saccharum officinarum. Ciencia y Tecnología, 15(1): 25-33. https://doi.org/10.18779/cyt.v15i1.540
[8] Rodas, M.G. (2021). Actividad antioxidante de la harina de mucílago de cacao (Theobroma cacao) para su aplicación en la agroindustria. Observatorio de la Economía Latinoamericana, 19(2): 107-123.
[9] Waluyo, J., Susastriawan, A.A.P., Purwanto, A. (2022). An effect of blending ratio on mechanical and thermal properties of the sawdust-cocoa pod briquettes. International Journal of Theoretical and Applied Mechanics, 7: 31-37.
[10] Arteaga Estrella, Y. (2013). Mucilago waste research in el Naranjal city. Revista ECA Sinergia, 4(4): 49-59. https://dialnet.unirioja.es/servlet/articulo?codigo=6197548.
[11] Villarroel-Bastidas, J.V., del Carmen Angulo-Ortega, G., Briones-Bitar, J. (2023). Reducing sugars degradation in

cocoa mucilage to produce bioethanol. Revista Facultad de Ingeniería Universidad de Antioquia, 108: 98-106. https://doi.org/10.17533/udea.redin.20220992

[12] Gutiérrez Rodas, M. (2021). Actividad antioxidante de la harina de mucílago de cacao (Theobroma cacao) para su aplicación en la agroindustria. Revista Observatorio de la Economía Latinomericana, 19(2): 107-123.

[13] Villa-Uvidia, D., Osorio-Rivera, M., Villacis-Venegas, N. (2020). Extraction, properties and benefits of mucilages. Dominio de las Ciencias, 6(2): 503-524. https://dialnet.unirioja.es/descarga/articulo/7398459.pdf.

[14] Ojo, A.O., de Smidt, O. (2023). Microbial composition, bioactive compounds, potential benefits and risks associated with Kombucha: A concise review. Fermentation, 9(5): 472. https://doi.org/10.3390/fermentation9050472

[15] Tejedor-Calvo, E., Morales, D. (2023). Chemical and aromatic changes during fermentation of Kombucha beverages produced using strawberry tree (arbutus unedo) fruits. Fermentation, 9(4): 326. https://doi.org/10.3390/fermentation9040326

[16] Halim, Y. (2023). Utilization of bay leaf tea in Kombucha production. Journal of Science and Technology, 7(1): 26-39.

[17] Wang, B., Rutherfurd-Markwick, K., Zhang, X.X., Mutukumira, A.N. (2022). Kombucha: Production and microbiological research. Foods, 11(21): 3456. https://doi.org/10.3390/foods11213456

[18] González-Téllez, S.V., Vázquez Olivares, D.A., Espinosa-Raya, J., Gómez-Pliego, R. (2019). Comparative study of the microbiota isolated from the Kombucha Mushroom and its use in the preparation of fermented foods for Metabolic Syndrome. Investigación y Desarrollo en Ciencia y Tecnología de Alimentos, 4. http://www.fcb.uanl.mx/IDCyTA/files/volume4/4/2/33.pdf.

[19] Coelho, R.M.D., de Almeida, A.L., do Amaral, R.Q.G., da Mota, R.N., de Sousa, P.H.M. (2020). Kombucha: Review. International Journal of Gastronomy and Food Science, 22: 100272. https://doi.org/10.1016/j.ijgfs.2020.100272

[20] Laavanya, D., Shirkole, S., Balasubramanian, P. (2021). Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. Journal of Cleaner Production, 295: 126454. https://doi.org/10.1016/j.jclepro.2021.126454

[21] Vázquez-Cabral, B.D., Moreno-Jiménez, M.R., Rocha-Guzmán, N.E., Gallegos-Infante, J.A., González-Herrera, S.M., Gamboa-Gómez, C.I., González-Laredo, R.F. (2016). Mexican oaks as a potential non-timber resource for Kombucha beverages. Revista Chapingo Serie Ciencias Forestales y Del Ambiente, 22(1): 73-86. https://doi.org/10.5154/r.rchscfa.2015.04.014

[22] Coelho, R.M.D., de Almeida, A.L., do Amaral, R.Q.G., da Mota, R.N., de Sousa, P.H.M. (2020). Kombucha. International Journal of Gastronomy and Food Science, 22: 100272. https://doi.org/10.1016/j.ijgfs.2020.100272

[23] Soares, M.G., de Lima, M., Schmidt, V.C.R. (2021). Technological aspects of Kombucha, its applications and the symbiotic culture (SCOBY), and extraction of compounds of interest: A literature review. Trends in Food Science & Technology, 110: 539-550. https://doi.org/10.1016/j.tifs.2021.02.017

[24] Kruk, M., Trząskowska, M., Ścibisz, I., Pokorski, P. (2021). Application of the "SCOBY" and Kombucha Tea for the production of fermented milk drinks. Microorganisms, 9(1): 123. https://doi.org/10.3390/microorganisms9010123

[25] Milanović, S.D., Lončar, E.S., Đurić, M.S., Malbaša, R. V., Tekić, M.N., Iličić, M.D., Duraković, K.G. (2008). Low energy Kombucha fermented milk-based beverages. Acta Periodica Technologica, 39: 37-46. https://doi.org/10.2298/APT0839037M

[26] Ayed, L., Ben Abid, S., Hamdi, M. (2017). Development of a beverage from red grape juice fermented with the Kombucha consortium. Annals of Microbiology, 67(1): 111-121. https://doi.org/10.1007/s13213-016-1242-2

[27] Xia, X., Dai, Y., Wu, H., Liu, X., Wang, Y., Yin, L., Wang, Z., Li, X., Zhou, J. (2019). Kombucha fermentation enhances the health-promoting properties of soymilk beverage. Journal of Functional Foods, 62: 103549. https://doi.org/10.1016/j.jff.2019.103549

[28] Kluz, M.I., Pietrzyk, K., Pastuszczak, M., Kacaniova, M., Kita, A., Kapusta, I., Zaguła, G., Zagrobelna, E., Struś, K., Marciniak-Lukasiak, K., Stanek-Tarkowska, J., Timar, A.V., Puchalski, C. (2022). Microbiological and physicochemical composition of various types of homemade Kombucha beverages using alternative kinds of sugars. Foods, 11(10): 1523. https://doi.org/10.3390/foods11101523

[29] Khaerah, A. (2023). Organoleptic test of Kombucha made from various tea sources and fermentation time. Bioeduscience, 7(1): 8-14. https://doi.org/10.22236/jbes/7110797

[30] Yang, H. (2023). Kombucha tea: Microorganisms communities composition, active metabolites, health functions and fermentation. Theoretical and Natural Science, 3: 780-788. https://doi.org/10.54254/2753-8818/3/20220481

[31] Gutiérrez Pulido, H., De la Vara Salazar, R. (2008). Experimental Analysis and Design. McGraw-Hill Publishing, Mexico. https://www.marcialpons.es/libros/analisis-y-diseno-de-experimentos/9786071507259/.

[32] NTE INEN-ISO 10523 Norma Técnica Ecuatoriana. Calidad de agua: Determinación del pH. https://docplayer.es/29853518-Nte-inen-iso-norma-tecnica-ecuatoriana-calidad-del-agua-determinacion-del-ph-iso-10523-2008-idt-primera-edicion.html, accessed on Apr. 11, 2023.

[33] INEN 971 Norma Técnica Ecuatoriana. Determinación de la Turbiedad, Método Nefelométrico. https://archive.org/details/ec.nte.0971.1984, accessed on Apr. 11, 2023.

[34] INEN 13 Norma Técnica Ecuatoriana. Leche: Determinación de la acidez titulable. https://archive.org/details/ec.nte.0013.1984, accessed on Apr. 11, 2023.

[35] INEN 273 Norma Técnica Ecuatoriana. Melazas: Determinación de la densidad de grados Brix. https://archive.org/details/ec.nte.0273.1990/page/n3/mode/2up, accessed on Apr. 11, 2023.

[36] NTE-INEN 340 norma técnica Ecuatoriana. Bebidas alcohólicas: Determinación del contenido de alcohol etílico. Método del alcoholímetro de vidrio. https://archive.org/details/ec.nte.0340.1994, accessed on Apr. 11, 2023.

[37] NTE-INEN 923:2013 norma técnica Ecuatoriana.

Materiales bituminosos. Determinación de la densidad por el método del picnómetro. https://archive.org/details/ec.nte.0923.1984/page/n1/mode/2up, accessed on Apr. 11, 2023.
[38] Water analysis - determination of metals by atomic absorption in natural, drinking, wastewaters, and wastewaters treated-test method. http://legismex.mty.itesm.mx/normas/AA/proy0909/proy-nmx-aa-051-1-scfi-08.pdf, accessed on Apr. 11, 2023.
[39] INEN 1529-7 Norma Técnica Ecuatoriana. Control microbiológico de los alimentos. Determinación de microorganismos coliformes. por la técnica de recuento de colonias. https://archive.org/details/ec.nte.1529.7.1990, accessed on Apr. 11, 2023.
[40] INEN 2608. (2019). Bebida de leche fermentada: Requisitos. https://archive.org/details/ec.nte.2608.2012, accessed on Jan. 5, 2023.
[41] NTE-INEN 1205: Agua. Determinación del número total de bacterias en placas. https://archive.org/details/ec.nte.1205.1985, accessed on Jan. 5, 2023.
[42] Maldonado-Jibaja, R., Carrillo-Herrera, P., Ramírez-Cárdenas, L., Carvajal-Larenas, F.E. (2018). Production of a fermented drink based on quinoa Chenopodium quinoa. Enfoque Ute, 9(3): 1-11. https://doi.org/10.29019/enfoqueute.v9n3.329
[43] Arguedas-Gamboa, P. (2014). Procedure definition to obtain a fermented drink from the coffee pulp. Tecnología en Marcha, 38-49. https://doi.org/10.18845/tm.v0i0.1654
[44] Dirzo, G.S., Ferrer, C.E.L., Valadez, M.F., Garfias, A.L.J., Rodríguez, J.A.A., Cruz, E.J.M., Chilpa, R.R. (2010). Estudio preliminar del Axokot, bebida tradicional fermentada, bajo una perspectiva transdisciplinaria. Investigación Universitaria Multidisciplinaria: Revista de Investigación de la Universidad Simón Bolívar, 9: 113-124.
[45] Apaza Mamani, V., Choque Mamani, R. (2018). Evaluation of the antioxidant activity and total polyphenols of a fermented beverage based on Globe Dark beet (Beta vulgaris L.) variety. Grade dissertation. Universidad Nacional de San Agustín. https://repositorioslatinoamericanos.uchile.cl/handle/2250/3264284.
[46] Sánchez-Muyulem, N., Erazo-Rodríguez, F., Almeida-Guzmán, M. (2022). Juice elaboration with three different fruits, adding 50% of Tibetan mushroom cultivation. Polo del Conocimiento: Revista científica, 7(1): 459-478. https://polodelconocimiento.com/ojs/index.php/es/article/view/3488/html.
[47] Velázquez-López, A., Covatzin-Jirón, D., Toledo-Meza, M.D., Vela-Gutiérrez, G. (2018). Fermented drink elaborated with lactic acid bacteria isolated from chiapaneco traditional pozol. CienciaUAT, 13(1): 165-178. https://doi.org/10.29059/cienciauat.v13i1.871
[48] Hernández-Monzón, A., Madernás-Sánchez, D., Pérez-Argüelles, R., Trujillo-Pérez, G., González-Góngora, I., Díaz-Abreu, J. (2019). Develop of a drink elaborated with rice flour and toasted sesame and ground (Sesamum indicum) and fermented with cultures probiotics. RTQ, 39: 90-104.
[49] Falcón-Romero, P., Aguirre-Vargas, E., Toscano-Leyva, A., Anate-Salazar, E. (2021). Elaboration and characterisation of a fermented drink made with the capulí fruit (Prunus serotina) and honey. Dominio de las Ciencias, 7(1): 59-78. https://dominiodelasciencias.com/ojs/index.php/es/article/view/1691/html.
[50] Rodríguez-Villacis, D., Hernández-Monzón, A. (2017). Develop a whey Drink Fermented with the addition of Aloe vera juice and Fruit Pulp. Tecnologia Quimica, 37(1): 46-57. https://www.redalyc.org/journal/4455/445552837005/.
[51] Arteaga Solórzano, R., Mendoza Rivadeneira, F., Vargas Zambrano, P., Barre Zambrano, R. (2019). Monitoring of the fermentative process of four fruit liqueurs (Passiflora edulis, Citrus cinensis, Citrus nobilis and Citrus maximus). Revista Arbitrada Interdisciplinaria Koinonía, 4(8): 752-764. http://doi.org/10.35381/r.k.v4i8.485
[52] Vásquez, C.M., Medina, C.M. (2010). Viscosidad cinemática y turbidez optimizadas en jugo mixto de "poro poro" y "caña de azúcar". UCV-Scientia, 2(1): 47-57. https://doi.org/10.18050/revucv-scientia.v2i1.831
[53] Ricardo, C., José, A.C.M., Yolanda, A., Gabriela, V.M. (2018). Efecto de la adición de quinua (Chenopodium quinoa wild) malteada y sin maltear en la elaboración de cerveza tipo Ale a base de cebada (Hordeum vulgare) malteada. Enfoque UTE, 9(2): 15-26. https://doi.org/10.29019/enfoqueute.v9n2.302
[54] Torres, B.J.A., Tello, C.M.E., Ostos, A.S. (2008). Development and optimization of an analytical methodology for sediment determination in a drink derived from the cacao. Revista Colombiana de Ciencias Químico-Farmacéuticas, 37(2): 177-190.
[55] Pastor, F.P., Galvañ, P.V., Belda, J.F. (2000). Contenido alcohólico de las bebidas "Sin alcohol". Adicciones, 12(3): 323-327. https://doi.org/10.20882/adicciones.643
[56] Gonzalez, R., Quiros, M., Morales, P. (2013). Yeast respiration of sugars by non-*Saccharomyces* yeast species: A promising and barely explored approach to lowering alcohol content of wines. Trends in Food Science & Technology, 29(1): 55-61. https://doi.org/10.1016/j.tifs.2012.06.015
[57] Verónica, R.A. (2011). Determinación de parámetros de fermentación para la producción de Kombucha utilizando una población mixta de microorganismos denominado fermento de té. Grade dissertation. Facultad de Ingeniería, Universidad Nacional Micaela Bastidas de Apurimac. https://alicia.concytec.gob.pe/vufind/Record/UNMB_603db30934359f4588605e0eb9b270d6.
[58] Bartolo, L., Cabellos, J., Gavidia, A., Hoyos, C. (2014). Effect of vacuum in absorbance and alcoholic strength intwo types of "Chicha de Jora". Agroindustrial Science, 3(2): 117-124. https://doi.org/10.17268/agroind.science.2013.02.05
[59] INEN 2395 Norma Técnica Ecuatoriana, Leches fermentadas: Requisitos. https://archive.org/details/ec.nte.2395.2011, accessed on Apr. 11, 2023.
[60] León, O.D.I., Méndez, C.D.S., Rodríguez, P.D.P., Puente, H.L., García, S.F.I., Salgado, B.R. (2012). Bromatological analysis and isolation of microorganisms with probiotic potential from pulque. Investigación Universitaria Multidisciplinaria, 1(11): 115-122.
[61] López, P., Zambrano, Á., Rosado, C., Peña, A. (2018).

Evaluation of a novel fermented beverage dairy based whey and sweet potato flour. La Técnica, 19: 47-60.
[62] NTE INEN 767. (2013). Carne y productos cárnicos. Determinación de mohos y levaduras. https://archive.org/details/ec.nte.0767.1985, accessed on Apr. 11, 2023.
[63] INEN-0765. (2013). Carne y productos cárnicos. Bacterias coliformes y escherichia coli. https://archive.org/details/ec.nte.0765.1985, accessed on Apr. 11, 2023.
[64] AOAC official methods of analysis. https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/, accessed on Mar. 26, 2023.
[65] Calvario-Palma, Á., Cruz-Hernández, J., Barroso-Bravo, L.D. (2019). Nutritional and sensory characterization of a muicle based beve rage that combines amaranto and berries. Revista Espamciencia, 10(2): 52-57.
[66] Marulanda, M., Granados, C., García-Zapateiro, L.A. (2016). Sensory analysis and physical-chemical estimation of the useful life of a yogurt beverage based on sweet whey fermented with Estreptococcus Salivarius ssp. Thermophilus and Lactobacillus Casei ssp. Casei. Producción+ Limpia, 11(1): 94-102.

NOMENCLATURE

SCOBY	Symbiotic Culture of Bacteria and Yeast
m.a.s.l.	Meters above sea level
cm	centimetre
℃	Celsius degrees
pH	Potential of hydrogen
INEN	Ecuadorian Standardization Service (by its acronym in Spanish)
V_{NaOH}	spent volume of 0.1 N sodium hydroxide
Pm	sample weight
rpm	revolution per minute
CFU	colony forming units
NTU	Nephelometric Turbidity Unit
g	gram
nm	nanometre
%	percentage