

Article

Physicochemical, Sensory, and Microbiological Evaluation of a Beverage Formulated from *Hibiscus sabdariffa* L., *Ananas comosus* L., and *Theobroma cacao* L. Mucilage

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Abstract

The study developed and characterized a natural beverage formulated from hibiscus extract (*Hibiscus sabdariffa* L.), pineapple (*Ananas comosus* L.), and cacao mucilage (*Theobroma cacao* L.), with the aim of valorizing agricultural by-products and providing a natural, healthy, and sustainable alternative. A completely randomized design with a 3×2 factorial arrangement was used. Six beverage formulations were prepared by combining three levels of Hibiscus extract–pineapple proportion (Factor A) with two levels of cacao mucilage (Factor B), resulting in six treatments that were evaluated in triplicate for their physicochemical, sensory, and microbiological properties. The analyses showed significant variations in °Brix (14.90–22.40), density (1.087–1.119 g/mL), and pH (2.71–3.31), indicating that cacao mucilage increased soluble solids retention and colloidal stability without altering acidity. In the sensory evaluation, treatment T1 (30% hibiscus extract, 10% pineapple, and 40% cacao mucilage) achieved the highest acceptance due to its balanced color, flavor, and texture. Microbiological analyses confirmed that the beverage complied with the limits established by the Ecuadorian standard NTE INEN 2337:2008 for non-carbonated fruit beverages. Overall, cacao mucilage proved to be an effective natural stabilizer that enhances the physicochemical and sensory quality of tropical beverages, representing an innovative strategy for the sustainable valorization of cacao by-products in the food industry.

Keywords: *Hibiscus sabdariffa*; *Ananas comosus*; *Theobroma cacao* L.; mucilage; beverage; physicochemical properties; sensory analysis



Academic Editors: Nenad Naumovski and Željko Dolijanović

Received: 23 November 2025

Revised: 13 April 2026

Accepted: 12 May 2026

Published: 3 July 2026

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1. Introduction

The consumption of natural beverages has increased significantly in recent years, driven by the search for healthy and sustainable alternatives that contribute to human well-being [1]. In Ecuador, traditional beverages based on plants and tropical fruits are an important part of the culinary culture, and hibiscus (*Hibiscus sabdariffa* L.) is one of the most appreciated ingredients due to its refreshing taste, characteristic acidity, and content of bioactive compounds with antioxidant and anti-inflammatory properties [2]. These compounds, mainly polyphenols and anthocyanins, contribute to the reduction in serum lipids and regulation of blood pressure, thereby promoting cardiovascular and metabolic health [2–7]. Consequently, its consumption has expanded from popular gastronomy to industrial formulations of natural beverages.

Hibiscus sabdariffa L., originally from Africa and currently cultivated in tropical regions of the Americas and Asia, has attracted growing scientific interest due to its therapeutic potential. Various studies have reported that *Hibiscus sabdariffa* L. extracts contain vitamins E and C, flavonoids, and phenolic acids with recognized antioxidant, anticancer, and cardioprotective activities [8,9]. However, the strongly acidic sensory profile of hibiscus may negatively affect consumer acceptance, which has motivated its combination with sweeter tropical fruits that provide gustatory balance and additional nutritional value. In this context, pineapple (*Ananas comosus* L.), recognized as the “queen of fruits”, stands out for its intense aroma and richness in bioactive compounds, bromelain, vitamin C, minerals, and fiber, which strengthen the immune system and favor digestion [10–12].

At the same time, cacao (*Theobroma cacao* L.), one of Ecuador’s main agricultural products, generates during postharvest a high-value by-product that remains underutilized: the pulp or cacao mucilage. This viscous material, which represents more than 70 L per ton of fruit, has a tropical flavor and is composed of natural sugars, pectins, fiber, and phenolic compounds [13–17]. In countries such as Brazil, Costa Rica, and Colombia, cacao mucilage is used in the production of beverages, jellies, and vinegars [18]. However, in Ecuador its utilization remains minimal due to lack of awareness (72%), low interest among farmers (22%), and limited technological innovation (6%) [14,18]. This situation contrasts with the growing interest in sustainable and circular-economy practices, where the integral use of agricultural by-products is a key pillar of modern agro-industrial development [19].

Despite recent advances in the study of ingredients, there are still discrepancies regarding the physicochemical behavior of cacao mucilage in liquid matrices. Previous studies have demonstrated that cacao mucilage improves viscosity and stability in fruit-based beverages due to its sugar and pectin content [20,21], whereas others suggest possible interactions with organic acids present in fruits such as hibiscus, which could modify the pH and final color of the product [4]. These controversies highlight the need to experimentally evaluate the synergy between compounds from Hibiscus extract, pineapple, and cacao mucilage, from both sensory and physicochemical–microbiological perspectives.

In this context, the aim of the present study was to develop an innovative beverage based on *Hibiscus sabdariffa* L., pineapple (*Ananas comosus* L.), and cacao mucilage (*Theobroma cacao* L.), and to evaluate its physicochemical, sensory, and microbiological properties. This approach explores the potential of cacao mucilage as a natural ingredient for the development of innovative beverages and contributes to the valorization of agro-industrial by-products in tropical regions [13,19].

2. Materials and Methods

2.1. Raw Materials and Origin

The raw materials used for beverage preparation were (*Hibiscus sabdariffa* L.), pineapple (*Ananas comosus* L.), and cacao mucilage (*Theobroma cacao* L.), selected based on their quality, local availability, and properties.

Hibiscus extracts were obtained from crops established in the cantons Buena Fe and Quevedo, Los Ríos province, Ecuador, regions characterized by a humid tropical climate and edaphoclimatic conditions favorable for this species. The calyces were harvested at physiological maturity and naturally shade-dried during post-harvest handling for approximately 3–5 days at ambient temperature. The dried calyces were then manually selected to remove remnants of sepals or foreign material and stored in amber glass containers with hermetic closures at 25 ± 2 °C, protected from light to prevent oxidation and pigment loss.

Pineapple cv. “Golden” was purchased from local markets in Santo Domingo de los Tsáchilas at commercial maturity, determined according to peel color index (4–5), fruit

firmness, and soluble solids content (12–14 °Brix), parameters commonly used to define optimal ripeness in pineapple intended for juice and beverage processing. Fruits were washed with potable water and disinfected with 50 ppm sodium hypochlorite for 15 min prior to processing.

Cacao mucilage was obtained from fresh *Theobroma cacao* L. pods (Nacional fine-flavor variety) from smallholder farms in Los Ríos province. The extraction was carried out immediately after pod opening, separating the seeds with pulp from the shell and allowing the material to rest for 12 h at ambient temperature so that the liquid fraction was released by gravity. The mucilage was then filtered and stored in sealed glass containers at 4 ± 1 °C until use. This procedure ensured the preservation of its natural sugars, phenolic compounds, and characteristic viscosity.

2.2. Preparation of the Hibiscus Extract

The hibiscus extract was prepared by infusion using 10 g of dried calyces per 500 mL of distilled water, equivalent to $20 \text{ g} \cdot \text{L}^{-1}$ (2% *w/v*). A total of 30 g of dried calyces were weighed and immersed in 1500 mL of distilled water preheated to 90 °C. The infusion was maintained for 15 min with occasional stirring to favor the diffusion of soluble compounds into the aqueous phase, following the methodology described by Pérez and Ortiz [2]. The extract was then filtered through Whatman No. 1 filter paper to remove solids and undissolved particles and allowed to cool to 25 °C. This simple and reproducible method avoids thermal degradation of anthocyanins and preserves the intense natural color of hibiscus [8]. The resulting extract showed a bright red color and a pH of approximately 2.4, suitable for combination with tropical fruit juices.

2.3. Pineapple Juice Extraction

Disinfected pineapples were peeled and cut into 2–3 cm pieces, ensuring complete removal of the fibrous core. Juice extraction was carried out using a domestic centrifugal juicer, which separates the liquid fraction from the fiber. The juice was passed through a 0.5 mm stainless steel sieve to remove coarse fibrous particles and large suspended solids naturally present in pineapple pulp before physicochemical analysis, ensuring a homogeneous liquid phase for subsequent measurements. Subsequently, the juice was stored in amber glass bottles at 4 °C until use.

Initial determinations showed an average pH of 4.1 ± 0.1 , titratable acidity of 0.54% (as citric acid), and soluble solids content of 13.2 ± 0.3 °Brix, confirming its suitability for developing beverages with a balanced sensory profile between sweetness and acidity [22]. Since the juice was not subjected to intense thermal treatment, its characteristic volatile compounds were preserved, enhancing the organoleptic profile of the final formulation.

2.4. Beverage Formulation and Preparation

Beverage formulations (T1–T6) were prepared on a *v/v* basis and standardized to 1 L of final beverage. The experiment followed a completely randomized design with a 3×2 factorial arrangement, where the hibiscus extract–pineapple juice proportion was considered Factor A (30:10, 20:20, and 10:30), and the cacao mucilage concentration was considered Factor B (40% and 35%). The complete composition of each formulation is presented in Table 1.

Formulations are expressed as percentage *v/v* and standardized to 1 L of final beverage. For each treatment, hibiscus extract, pineapple juice, and cacao mucilage were mixed, and distilled water was added to complete the final volume before homogenization and pasteurization. The beverages were then pasteurized in a thermostatic water bath (Memmert GmbH, Schwabach, Germany) at 80 °C for 15 min, which reduced the microbial load without significantly affecting the sensory and bioactive properties of the ingredients.

After pasteurization, samples were filled into 250 mL glass bottles previously sterilized and stored at 4 ± 1 °C until analysis.

Table 1. Complete formulation of the beverage treatments expressed per 1 L of final beverage.

Treatment	Hibiscus Extract (%)	Pineapple Juice (%)	Cacao Mucilage (%)	Distilled Water (%)
T1	30	10	40	20
T2	30	10	35	25
T3	20	20	40	20
T4	20	20	35	25
T5	10	30	40	20
T6	10	30	35	25

The percentages shown in Table 1 correspond to the proportion of each ingredient in the final beverage formulation. Distilled water was added to complete the final beverage volume (1 L).

2.5. Physicochemical Analysis

Physicochemical determinations were performed in triplicate for each treatment following AOAC (2020) official methods [23]. The pH was measured using a digital potentiometer (Hanna Instruments, Woonsocket, RI, USA) calibrated with pH 4.00 buffer solutions before each set of measurements.

Titrate acidity was determined by titrating with 0.1 N NaOH using phenolphthalein as indicator and expressed as percentage of citric acid. Total soluble solids were measured with a digital refractometer (Atago Co., Ltd., Tokyo, Japan) and expressed in °Brix. Density was determined by pycnometry at 25 °C. Color parameters (L^* , a^* , b^*) were measured according to the CIELAB color space recommendations [24].

2.6. Microbiological Methods

Microbiological analysis was performed on the selected beverage formulation (T1), which showed the highest sensory acceptance and was considered the most suitable formulation for potential product development. Product safety was evaluated according to the Ecuadorian Standard INEN 1529-2013 [25], which establishes the microbiological methods for non-carbonated beverages.

Total mesophilic aerobic bacteria were enumerated using Plate Count Agar (PCA) after incubation at 35 ± 2 °C for 48 h. Total and fecal coliforms were determined using Violet Red Bile Agar (VRBA) and incubated at 35 ± 2 °C for 24 h. Molds and yeasts were enumerated on Potato Dextrose Agar (PDA) after incubation at 25 °C for 5 days. The presence of *Escherichia coli* was investigated using selective culture methods according to the procedures established in the Ecuadorian Standard INEN 1529-2013 [25]. All analyses were performed in triplicate, and results were expressed as colony-forming units per milliliter (CFU/mL).

2.7. Sensory Evaluation

The sensory panel consisted of 25 semi-trained assessors aged between 20 and 45 years who were regular consumers of fruit-based beverages. Panelists who evaluated color, aroma, flavor, texture, and overall acceptance using a five-point hedonic scale (1 = dislike very much; 5 = like very much). The sensory analysis procedure followed standard sensory evaluation guidelines [26,27]. Samples were identified with random three-digit codes and presented in randomized order to avoid bias. Data were recorded and statistically analyzed to identify significant differences among treatments as a function of mucilage content. Sensory data obtained from the five-point hedonic scale were analyzed using analysis of variance (ANOVA), which is commonly applied in sensory studies when the number of

panelists is adequate and the data distribution approximates normality. When significant differences were detected, Tukey's test was used for mean comparison ($\alpha = 0.05$).

2.8. Statistical Analysis

The statistical analysis followed a completely randomized design with a 3×2 factorial arrangement, considering two experimental factors and three levels for the first factor. Factor A corresponded to the proportions of hibiscus extract and pineapple juice, with three levels: 30% hibiscus extract + 10% pineapple juice, 20% hibiscus extract + 20% pineapple juice, and 10% hibiscus extract + 30% pineapple juice. Factor B corresponded to the percentage of cacao mucilage, with two levels: 40% and 35%. Thus, six experimental treatments were obtained, each with three replicates, for a total of eighteen experimental units.

Independent variables were the levels of factors A and B. Dependent variables included sensory parameters (color, aroma, flavor, texture), physicochemical parameters (pH, °Brix, density), and microbiological parameters (counts of *Escherichia coli*, molds, and yeasts). These variables were selected due to their relevance for determining the quality, stability, and safety of natural beverages.

Data were expressed as mean $\pm$ standard deviation. Statistical analyses were performed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was applied to evaluate the effects of Factor A, Factor B, and their interaction ($A \times B$). When significant differences were detected, treatment means were compared using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

2.9. Ethical Considerations and Use of Generative Artificial Intelligence

The study involved a sensory evaluation conducted with voluntary adult participants. All panelists were informed about the objectives of the study and provided informed consent prior to participation. The sensory evaluation represented minimal risk, as it involved the tasting of a non-alcoholic beverage prepared from commonly consumed food ingredients. The study was approved by the Research and Ethics Committee of the Quevedo State Technical University. The research does not affect living organisms such as human beings, in accordance with the Regulations for the Approval and Monitoring of Research Ethics Committees for Human Subjects (CEISH), published in Supplement No. 279 of the Official Registry on 1 July 2014. Approval Code: CERT- ÉTICA-004-DICYT-2026.

During manuscript preparation, the authors used the generative artificial intelligence tool ChatGPT (OpenAI, GPT-5.5 version, San Francisco, CA, USA) exclusively for language editing, style correction, and adaptation of the text to the journal's editorial format. No artificial intelligence was used in the experimental design, data collection, or interpretation of results. The authors have reviewed and edited all AI-assisted content and take full responsibility for the final version of the manuscript.

2.10. Data and Materials Availability

The experimental data generated and analyzed in this study are available from the corresponding author upon reasonable request. There are no restrictions on access to the materials or the experimental protocols described, ensuring reproducibility of the work.

3. Results and Discussion

3.1. Physicochemical Properties of the Hibiscus–Pineapple–Cacao Mucilage Beverage

Table 2 summarizes the physicochemical results of the six formulations (T1–T6) prepared with different proportions of hibiscus extract, pineapple, and cacao mucilage. Mean values of °Brix ranged from 14.90 to 22.40, density from 1.087 to 1.119 g/mL, and pH from

2.71 to 3.31, reflecting the direct influence of formulation on solute concentration, acidity, and beverage texture.

Table 2. Physicochemical properties of the beverage formulations (T1–T6), including °Brix, density, and pH.

Treatment	°Brix	Density (g/mL)	pH	Titrateable Acidity
T1	19.83 ± 0.23 ^b	1.107 ± 0.001 ^b	2.90 ± 0.02 ^c	0.22 ± 0.01 ^b
T2	19.03 ± 0.15 ^{bc}	1.104 ± 0.001 ^c	2.86 ± 0.01 ^d	0.23 ± 0.01 ^b
T3	18.10 ± 0.17 ^{cd}	1.107 ± 0.001 ^b	3.04 ± 0.00 ^b	0.16 ± 0.00 ^a
T4	18.33 ± 0.06 ^{cd}	1.102 ± 0.000 ^{cd}	3.08 ± 0.00 ^b	0.20 ± 0.00 ^b
T5	22.40 ± 0.10 ^a	1.119 ± 0.001 ^a	3.31 ± 0.00 ^a	0.13 ± 0.00 ^a
T6	17.83 ± 0.90 ^d	1.101 ± 0.002 ^d	2.84 ± 0.03 ^d	0.26 ± 0.02 ^c

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters within each column indicate significant differences among treatments according to Tukey's test ($p < 0.05$).

Total soluble solids fluctuated between 17.83 and 22.40 °Brix, with a progressive increase as pineapple and mucilage proportions rose. T1 (19.83 °Brix) and T5 (22.40 °Brix) showed the highest levels, evidencing the contribution of pineapple sugars and the capacity of cacao mucilage to retain dissolved solids. Cacao mucilage typically contains 10–15% total sugars and soluble pectins that increase viscosity and system stability [21,28]. These values are within the range reported for tropical pineapple-based beverages (18–22 °Brix) [29], suggesting that the combination of pineapple and cacao mucilage favors soluble solids concentration and sensory balance without added sucrose.

Density ranged from 1.101 to 1.119 g/mL, showing a direct relationship with °Brix. T5 (1.119 g/mL) exhibited the highest density, whereas T6 (1.101 g/mL) showed the lowest value. This behavior is attributed to the higher concentration of solutes and polysaccharides contributed by cacao mucilage, which increases specific mass and structural cohesion [28]. Densities between 1.10 and 1.12 g/mL are considered adequate for natural beverages, as they provide body and colloidal stability [28,29]. In this formulation, cacao mucilage acts as a natural stabilizer by reducing sedimentation and maintaining product homogeneity, demonstrating its potential as a substitute for synthetic hydrocolloids.

The pH values (2.84–3.31) showed an inverse trend with respect to hibiscus extract content. Treatments with higher hibiscus proportions tended to be more acidic, whereas formulations containing higher proportions of pineapple and mucilage showed slightly higher pH values. This behavior can be explained by the presence of organic acids (hibiscus, citric, and malic acids) in hibiscus and the buffering effect of the neutral carbohydrates present in mucilage [29]. The pH range obtained (2.5–3.5) is considered optimal for hibiscus-based beverages, ensuring microbiological stability and a balanced taste profile [30,31]. Among the evaluated formulations, T4 (pH 3.08) presented a suitable balance between acidity and sweetness, which may contribute to improved sensory acceptance.

Titrateable acidity values ranged from 0.13 to 0.26% (citric acid equivalents), showing an inverse relationship with pH values. Treatments with higher hibiscus proportions exhibited greater acidity due to the presence of organic acids naturally occurring in *Hibiscus sabdariffa* L., such as hibiscus, citric, and malic acids. These values fall within the typical range reported for hibiscus-based beverages and contribute to microbiological stability and sensory balance.

Colorimetric analysis revealed significant differences among treatments in the CIELab parameters (Table 3). Luminosity (L^*) values ranged from 36.41 to 39.42, indicating moderately dark beverages characteristic of hibiscus-based formulations. Positive a^* values confirmed the predominance of red tones associated with anthocyanins from *Hibiscus sabdariffa*, while positive b^* values indicated a secondary yellow component contributed by

pineapple pigments. Treatment T1 exhibited significantly higher values, suggesting greater red color intensity compared with the other formulations.

Table 3. Colorimetric parameters (CIELab) of the beverage formulations.

Treatment	L*	a*	b*
T1	38.47 ± 5.52 ^c	27.56 ± 0.02 ^b	16.50 ± 0.03 ^b
T2	37.69 ± 8.38 ^{ab}	23.18 ± 0.01 ^a	17.25 ± 0.02 ^a
T3	36.41 ± 2.92 ^a	21.48 ± 0.02 ^a	16.36 ± 0.01 ^a
T4	39.42 ± 1.55 ^b	23.45 ± 0.01 ^a	17.46 ± 0.01 ^a
T5	37.19 ± 2.68 ^{ab}	22.49 ± 0.01 ^a	17.36 ± 0.01 ^a
T6	37.06 ± 3.22 ^a	22.71 ± 0.01 ^a	16.61 ± 0.03 ^a

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$).

ANOVA tables for °Brix, density, and pH (Tables 4 and 5) showed that both main factors—hibiscus/pineapple proportion (Factor A) and cacao mucilage percentage (Factor B)—as well as their interaction ($A \times B$) had statistically significant effects ($p < 0.05$) on these variables. For °Brix, Factor B showed the highest F-value, confirming that mucilage has the strongest effect on soluble solids concentration. Similarly, its high F-value in the density model indicates that cacao mucilage is the main determinant of beverage body and structure, followed by the hibiscus–pineapple ratio, which modifies sugar content and consistency.

Table 4. Analysis of variance (ANOVA) for soluble solids (°Brix) in the formulations of the beverage based on hibiscus flower, pineapple and cacao mucilage.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Main effects					
Factor A: Hibiscus extract/Pineapple	11.1144	2	5.55722	36.11	0.0000
Factor B: percentage of cacao mucilage	13.1756	1	13.1756	85.62	0.0000
Interactions					
AB	19.1478	2	9.57389	62.21	0.0000
Error	1.84667	12	0.153889		
Total (corrected)	45.2844	17			

Table 5. Analysis of variance (ANOVA) for pH values in the beverage formulations based on hibiscus flower, pineapple, and cacao mucilage.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Main effects					
Factor A: Hibiscus extract/Pineapple	0.142711	2	0.0713556	267.58	0.0000
Factor B: percentage of cacao mucilage	0.112122	1	0.112022	420.08	0.0000
Interactions					
AB	0.220311	2	0.110156	413.08	0.0000
Error	0.0032	12	0.000266667		
Total (corrected)	0.478244	17			

The significant interaction between pineapple and mucilage contents suggests a synergistic effect that favors the retention of dissolved solids and improves texture. Moreover, the low residual variability in the ANOVA models indicates high experimental precision and an adequate statistical fit. From a technological perspective, these results

confirm that increasing mucilage content and optimizing the hibiscus–pineapple balance allow fine control of °Brix, density, and pH, which are key parameters for natural beverage formulation.

From a technological and industrial perspective, the standardization of raw materials and process conditions is essential for the reproducible manufacture of this beverage. For cacao mucilage, basic incoming quality control should include soluble solids (°Brix), pH, absence of visible impurities, filtration conditions, and refrigerated storage prior to use. Likewise, pineapple juice and hibiscus extract should be standardized in terms of maturity, pH, and soluble solids to reduce formulation variability. At the product level, target specifications may include a pH range of 2.8–3.3, soluble solids between 18 and 22 °Brix, and density values around 1.10–1.12 g/mL, together with stable color attributes. Process control should also consider verification of pasteurization conditions, hygienic filling, and refrigerated storage to preserve product quality and microbiological stability. Although these specifications were not validated at industrial scale in the present study, they provide a useful basis for future scale-up and process standardization.

3.2. Sensory Analysis

Figure 1 presents the overall sensory profile of the formulations, as evaluated by the 25 semi-trained panelists for the attributes color, aroma, flavor, texture, and overall acceptance. There was a clear preference for T1 and, to a lesser extent, T6, whereas T3 and T4 received the lowest scores. These results confirm that the combination of 30% hibiscus extract, 10% pineapple, and 40% cacao mucilage yields a sensorially balanced beverage, with harmony between sweetness, acidity, texture, and visual appearance.

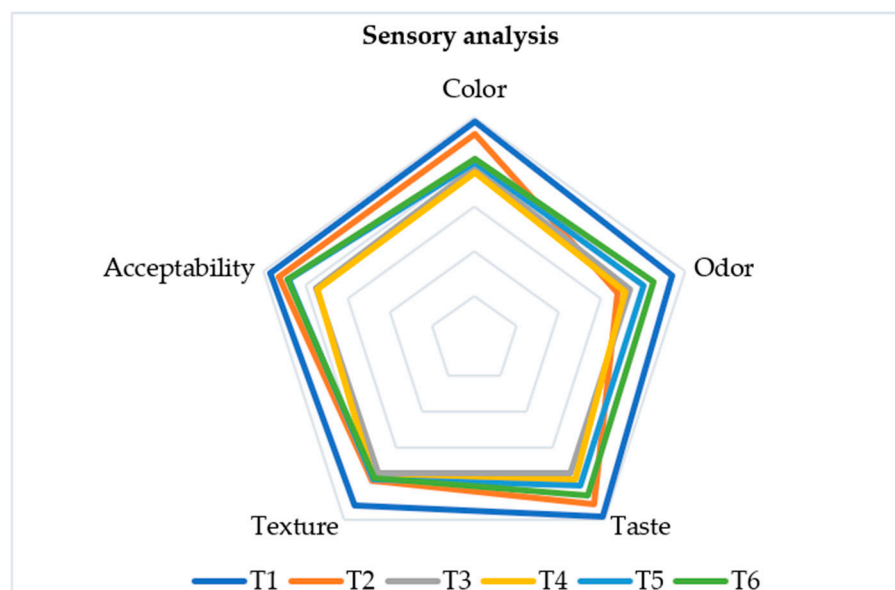


Figure 1. Sensory acceptability of the beverage formulations based on the mean scores of 25 semi-trained panelists using a five-point hedonic scale.

For color, T1 achieved the highest score (5.0), indicating a bright and stable red hue perceived as more attractive by panelists. This behavior is due to the anthocyanins present in hibiscus, whose stability is favored by the acidic pH and the protective effect of mucilage polysaccharides. Several studies have shown that adding natural biopolymers improves color stability by protecting pigments against oxidation and thermal degradation [31–34]. Carotenoids and volatiles from pineapple also contribute to product brightness and a more intense, natural visual perception [35].

Regarding aroma, T1 was again the best-rated treatment, with a balanced aromatic profile. The combination of organic acids from hibiscus and fruity esters from pineapple produced a fresh, tropical aroma. This is consistent with reports indicating that blends of tropical fruits and floral extracts can enhance the release of pleasant volatile notes [36]. Additionally, mucilage, rich in sugars and proteins, may act as a carrier that prolongs aroma perception during tasting [37].

In terms of flavor, panelists highlighted T1 for its gustatory balance between hibiscus acidity and pineapple sweetness, complemented by simple sugars from cacao mucilage. This provided a refreshing and rounded sensation without excessive acidity or astringency. Similar behavior has been reported in fruit-based formulations where the interaction between acidic matrices and polysaccharides generates more stable and pleasant flavor profiles [38]. Furthermore, cacao mucilage has been shown to enhance sweetness perception and stability in natural beverages without requiring added sweeteners [21].

Texture analysis showed that incorporating 40% cacao mucilage improved body and homogeneity, yielding a smooth, fluid beverage without excessive viscosity. This behavior is explained by the capacity of cacao mucilage to form a stable colloidal structure at relatively low concentrations, as reported in plant-based beverages [20]. Likewise, soluble fiber in these matrices contributes to creamier and more uniform textures, increasing sensory acceptance [7,39].

Overall acceptance reached its maximum value in T1 (4.9 ± 0.2), confirming that the interaction among the three ingredients was decisive for positive consumer perception. The synergy between hibiscus phenolics, pineapple sugars, and mucilage pectins produced a balanced, fresh beverage with a harmonious sensory profile. These findings are consistent with reports indicating that combining hibiscus with tropical fruits enhances consumer acceptance due to its balanced flavor and characteristic aroma [3]. Additionally, using cacao mucilage as a plant-based by-product reinforces perceptions of sustainability and naturalness, attributes increasingly valued in products [40].

3.3. Microbiological Analysis

The microbiological assays were carried out to assess the effectiveness of thermal treatment and to ensure the sanitary quality of the formulations. The hibiscus–pineapple–mucilage beverage showed adequate microbiological behavior, fully complying with the limits established by NTE INEN 2337:2008 for non-carbonated fruit beverages [36]. The microbiological quality of the beverage formulations is summarized in Table 6.

Table 6. Microbiological quality of the selected beverage formulation002E.

Microorganism	Result (CFU/mL)
Mesophilic aerobic bacteria	<10
Total coliforms	<10
Molds	<10
Yeasts	<10
<i>Escherichia coli</i>	Not detected

Results expressed as CFU/mL. Detection limit of the method: 10 CFU/mL.

3.3.1. Molds and Yeasts

Microbiological analysis was performed for the selected formulation (T1), which showed the highest sensory acceptance. The results indicated that mesophilic aerobic bacteria, total coliforms, molds, and yeasts were below the detection limit of the method (<10 CFU/mL), while *Escherichia coli* was not detected. These results confirm the microbiological quality of the beverage and its compliance with the limits established by the Ecuadorian standard NTE INEN 2337:2008 for non-carbonated fruit beverages.

The low microbial counts can be attributed to good hygienic practices during processing and, mainly, to the pasteurization treatment applied at 80 °C for 15 min, which effectively reduced the initial microbial load. In addition, the acidic pH of the beverage (≈ 3.0) and the presence of phenolic compounds and anthocyanins from hibiscus contribute to creating an unfavorable environment for the growth of spoilage microorganisms. This antimicrobial effect has been previously documented in beverages prepared from *Hibiscus sabdariffa*, where organic acids and polyphenolic compounds inhibit fungal and bacterial growth [41,42].

Cacao mucilage contributes natural sugars and polysaccharides with humectant capacity; however, its interaction with acidic compounds and soluble fibers may limit microbial proliferation by reducing water activity and creating a less favorable environment for microbial development [43]. These factors contribute to maintaining the microbiological stability of the beverage under appropriate processing and storage conditions.

3.3.2. *Escherichia coli*

The detection of *E. coli* was also performed to verify the absence of fecal contamination. The results showed no presence of *E. coli* in any of the analyzed samples, ensuring compliance with Ecuadorian safety standards for non-carbonated beverages [44]. This finding confirms that processing, handling, and packaging conditions were appropriate, and that pasteurization was effective in eliminating potential pathogenic microorganisms.

The absence of *E. coli* is a key indicator of product safety, reflecting good manufacturing practices during preparation, pasteurization, and storage. Studies on natural beverage produced from plant extracts report similar results, highlighting the combined role of temperature, pH, and phenolic compounds in inhibiting bacterial growth [45,46].

Additionally, the bioactive composition of cacao mucilage may exert a mild complementary antimicrobial effect [47]. In summary, microbiological results confirm that the developed beverage meets international quality and safety standards and is suitable for human consumption. The observed stability not only guarantees product safety but also supports the feasibility of scaling up its production while maintaining sensory and quality.

From a sustainability and scalability perspective, the processing steps used in this study (aqueous extraction by infusion, homogenization, and batch pasteurization) are compatible with conventional beverage manufacturing technologies. At industrial scale, these operations could be optimized by implementing energy-efficient heating systems, heat recovery during pasteurization, and improved water management strategies. The use of cacao mucilage as a raw material also contributes to circular economy principles by valorizing an agro-industrial by-product that is commonly discarded during cacao processing.

Future studies may also explore process intensification approaches aimed at improving extraction efficiency and reducing energy consumption. Emerging technologies such as hydrodynamic cavitation have been reported as promising green extraction methods capable of enhancing mass transfer, improving homogenization, and reducing processing time and energy demand in beverage processing systems [48]. These approaches may contribute to better process standardization and industrial scalability of hibiscus–fruit beverages.

4. Conclusions

This study demonstrated that it is technologically feasible to formulate a natural beverage from *Hibiscus sabdariffa* L., *Ananas comosus* L., and *Theobroma cacao* L. mucilage, and that the proportions of these ingredients have a significant effect on its physicochemical, sensory, and microbiological characteristics. The physicochemical analysis revealed that

increasing the proportion of cacao mucilage enhanced soluble solids, density, and texture, while maintaining pH values within the optimal range for stability. Sensory evaluation showed that formulation T1 (30% hibiscus extract, 10% pineapple, and 40% cacao mucilage) achieved the highest acceptance due to its balanced color, flavor, aroma, and texture. Microbiological analyses confirmed the absence of *Escherichia coli*, molds, and yeasts, indicating that the thermal treatment and intrinsic acidity ensured product safety according to NTE INEN 2337:2008.

Overall, the results confirm that cacao mucilage functions as an effective natural stabilizer capable of improving beverage quality while promoting sustainable use of agro-industrial by-products. The beverage developed in this study presents strong technological and commercial potential for the food sector. Future research should focus on evaluating shelf life, stability of bioactive compounds, and consumer acceptance under different storage and processing conditions.

5. Limitations

This study has some limitations that should be considered. The beverage formulations were evaluated at laboratory scale, and industrial-scale processing conditions were not assessed. In addition, microbiological analysis was performed on the selected formulation with the highest sensory acceptance, while future studies should evaluate all formulations under extended storage conditions. Shelf-life stability, bioactive compound retention, and consumer acceptance with a larger and more diverse panel were not fully explored. Therefore, further research is recommended to evaluate storage stability, scale-up feasibility, and broader consumer perception of the developed beverage.

Author Contributions: Conceptualization, R.R.-C. and J.M.-S.; methodology, R.R.-C. and D.M.-T.; software, J.M.-S.; validation, R.R.-C., J.M.-S. and G.P.-L.; formal analysis, J.M.-S.; investigation, R.R.-C., D.M.-T., C.D.C. and G.P.-L.; resources, Á.F. and R.R.-C.; data curation, D.M.-T. and G.P.-L.; writing—original draft preparation, R.R.-C., D.M.-T. and G.P.-L.; writing—review and editing, R.R.-C., J.M.-S. and Á.F.; visualization, R.R.-C.; supervision, J.M.-S.; project administration, R.R.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fondo Competitivo de Investigación, Ciencia y Tecnología (FOCICYT 2024–2025) of the Universidad Técnica Estatal de Quevedo (UTEQ), Ecuador, through the Tenth Competitive Research Call. The study was supported by institutional resources from the competitive research program of the Ninth and Tenth FOCICYT Calls. Specifically, this work is part of the project titled “Optimización nutricional mediante el uso estratégico del mucílago de cacao (*Theobroma cacao* L.) y frutas tropicales en el diseño de productos innovadores,” approved under the Tenth FOCICYT Competitive Research Call.

Institutional Review Board Statement: The sensory evaluation involved voluntary participation of adult panelists. The study followed institutional guidelines for minimal-risk food sensory evaluation studies and did not require formal approval from an institutional ethics committee. The study was approved by the Research and Ethics Committee of the Universidad Técnica Estatal de Quevedo (UTEQ), Ecuador. The research does not affect living organisms such as human beings, in accordance with the Regulations for the Approval and Monitoring of Research Ethics Committees for Human Subjects (CEISH), published in Supplement No. 279 of the Official Registry on 1 July 2014. Approval Code: CERT-ÉTICA-004-DICYT-2026.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the sensory evaluation.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank the Universidad Técnica Estatal de Quevedo for technical and logistical support, particularly the Food Analysis Laboratory of the Faculty of Industrial and Production Sciences for providing equipment and infrastructure for physicochemical, microbiological, and sensory analyses. The authors also acknowledge local producers from Los Ríos province for supplying the raw materials used in this study. During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GPT-5.5, San Francisco, CA, USA) solely for language editing and improvement of style. The authors have reviewed and edited all content and take full responsibility for the final version of the article.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviation

The following abbreviation is used in this manuscript:

CFU Colony-forming units

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