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# Incorporation of the fruit of *Parmentiera aculeata* as a low-cost alternative flavoring for the formulation of a fermented beverage analogue of kombucha

# Incorporación del fruto de *Parmentiera aculeata* como saborizante alternativo de bajo costo para la formulación de una bebida fermentada análoga de kombucha

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## ABSTRACT

"Kombucha" is a traditional fermented beverage from the Asian continent related to important biological activities and for its content of microorganisms. The beverage is acidic and for consumers, it is not pleasant. Therefore, it has been decided to flavor the beverage with fruits and vegetables to improve its sensory characteristics through a second fermentation. The objective is to develop an analogous kombucha beverage by incorporating the *Parmentiera aculeata* fruit as a low-cost alternative flavoring, aiming at its valorization. The kombucha beverage was created by a 12-day fermentation and through a randomized complete design, it was flavored with *P. aculeata* juice, fruit pieces, and both in a second fermentation. pH, °Brix, and titratable acidity (lactic acid and acetic acid, g/L) were monitored. Mainly, pH values consistent with those previously reported (3.0-3.5), 1 °Brix consumption during the first fermentation, and titratable acidity lower than previous reports were observed. Formulation 3 was rated as "I like it a little". This is the first report of the *P. aculeata* fruit usage in the flavoring of a kombucha analog beverage and is an encouragement for developing new fermented beverages.

**KEY WORDS:** Fermented beverages, health drinks, kombucha, probiotics, SCOBY.

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**RESUMEN**

La “kombucha” es una bebida fermentada tradicional del continente asiático relacionada con actividades biológicas importantes y por su contenido de microorganismos. La bebida es ácida y para los consumidores no es agradable. Por lo que, se ha optado por saborizar la bebida con frutas y hortalizas para mejorar sus características sensoriales mediante una segunda fermentación. El objetivo es desarrollar una bebida análoga de kombucha con la incorporación del fruto de *Parmentiera aculeata* como saborizante alternativo de bajo costo pretendiendo la valorización del mismo. Se desarrolló la bebida de kombucha por fermentación de 12 días y mediante un diseño completo al azar, se saborizó con jugo de *P. aculeata*, trozos del fruto y ambos en una segunda fermentación. Se monitoreó pH, °Brix y acidez titulable (ac. láctico y ac. acético, g/L). Principalmente, se observaron valores de pH consistentes con los reportados previamente (3.0-3.5), el consumo de 1 °Brix durante la primera fermentación y una acidez titulable inferior a los reportes previos. La formulación 3 fue calificada como “me gusta poco”. Este es el primer reporte del uso del fruto de *P. aculeata* en la saborización de una bebida análoga de kombucha y es un aliciente para el desarrollo de nuevas bebidas fermentadas.

**PALABRAS CLAVE:** Bebidas fermentadas, bebidas saludables, kombucha, probióticos, SCOBY.

## Introduction

The sedentary lifestyle and poor eating habits of the population, driven by food industry trends, have led to a deterioration in global health. This has manifested in diseases such as obesity, hypertension, and diabetes mellitus, which are factors that increase the mortality risk in COVID-19 patients (Navarrete-Mejía *et al.,* 2020). Currently, there are several tedious and expensive treatments to combat cardiovascular diseases. However, there is a growing interest in healthy alternatives, and a significant increase has been observed in the market for healthy beverages, including kombucha (Mordor Intelligence Research & Advisory, 2023). The market size for kombucha is estimated at $2.97 billion and is expected to grow to $4.65 billion by 2029, representing a compound annual growth rate of 9.48 % during the 2024 to 2029 period. It is important to highlight that this beverage can be found in the market as “original” kombucha and flavored kombucha, which includes herbs, spices, flowers, and fruits in its formulation (Mordor Intelligence Research & Advisory, 2024). Kombucha is a non-alcoholic refreshing beverage obtained by fermenting black tea (fermented), water, sugar, and bacteria and yeast communities, called Symbiotic Culture of Bacteria and Yeasts (SCOBY) (Laureys *et al.,* 2020). Other authors mention the use of green tea (non-fermented) and oolong tea (semi-fermented) to produce the beverage (Júnior *et al.,* 2022). The microbial consortium used in this beverage production includes acetic acid bacteria (*Komagataeibacter spp., Gluconobacter spp*., *Gluconoacetobacter spp*., and *Acetobacter spp.*), lactic acid bacteria (*Lactobacillus spp., Lactococcus spp.,* and *Leuconostoc spp.*) (Antolak *et al.,* 2021), and yeasts (*Saccharomyces, Zygosaccharomyces, Brettanomyces, Schizosaccharomyces, Candida*, and *Dekkera*) (Anantachoke *et al.,* 2023; Barakat *et al.,* 2023). Several reports have mentioned that kombucha is a functional beverage. It acts as a digestive system regulator, contains a considerable amount of antioxidants, keeps the skin healthy, and aids in arthritis treatment due to its high glucosamine content. This promotes the production of hyaluronic acid in the body, thus preserving the cartilage structure and reducing joint pain (Ricaurte Heredia, 2020).

Recent research on kombucha has focused on studying the dynamics of microorganisms, the production of metabolites with biological activity, and the influence of the beverage on human health. Additionally, some studies have aimed to improve its sensory qualities by adding different ingredients. As a result, there is a strong trend towards incorporating fruits into kombucha to enhance its organoleptic characteristics and nutritional and functional properties. Ingredients used to flavor the beverage include mango, rosemary, snake fruit, lavender, oregano, and fennel, among others (Fernández Ormaza & Muñoz Jiménez, 2022; Tapias *et al.,* 2022; Luvison *et al.,* 2023). However, the impact of *P. aculeata* fruit juices during fermentation with the kombucha consortium is not well understood. This fruit is high in carbohydrates (sugars and fiber), and vitamins (C and E), and possesses several health benefits, including hypoglycemic, anti-urolithic, antimicrobial, and antioxidant activities (Andrade-Cetto & Heinrich, 2005; Estanislao-Gómez *et al.,* 2016; Ibarra-Morales *et al.,* 2021; Morales-Sánchez *et al.,* 2015; Pérez *et al.,* 2000). Additionally, *P. aculeata* is a Mexican native plant with a wide distribution throughout the territory, year-round fruit availability, and a significant presence in traditional Mexican medicine (Jiménez-Osornio, 2018). A study in this area would provide an alternative product for health-conscious consumers and add value to the fruit by using it as a low-cost alternative flavoring. Therefore, the objective of the present work is to use the *P. aculeata* fruit to flavor a fermented kombucha-type beverage, including an acceptability analysis and chemical profile of this beverage.

## Material and Methods

### Chemical reagents and culture media

All chemical reagents used in this study were purchased from Fermont (Monterrey, Mexico) and Jalmek Científica (San Nicolás de los Garza, Mexico). For microbiological analysis, standard count agar and red violet bile lactose agar were purchased from BD Bioxon (Estado de México, Mexico), and potato dextrose agar was purchased from Difco Laboratories (Detroit, Michigan).

### Collection, selection, and vegetal material preparation

The *P. aculeata* fruit was collected from various areas of Ciudad Valles, San Luis Potosí estate, selecting fruits at ripening levels 3-4 according to the color scale reported by Angón-

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Galván (2006). The selected fruits were taken to the Food Analysis Laboratory of the Tecnológico Nacional de México/IT of Ciudad Valles, where they were washed and disinfected with a 3 % solution of colloidal silver proteins. The juice used in this study was obtained by mechanical extraction, followed by filtering and pasteurization at 80 °C for 15 seconds. The juice was preserved in previously sterilized bottles and kept refrigerated until use. It was also subjected to microbiological analysis to ensure its safety following the Mexican Official Standards; determining aerobic mesophilic bacteria (NOM-092-SSA1-1994), total coliforms (NOM-113-SSA1-1994), and molds and yeasts (NOM-111-SSA1-1994).

### Traditional kombucha brewing: First fermentation

First, a black tea infusion (9 g/L of boiled water) was prepared, allowed to stand for 10 min, and filtered into a sterile flask. Then, 1 L of water and 60 g of table sugar were added. Subsequently, SCOBY (approximate weight of 10 g, wet basis) and 250 mL of starter culture were added. The flask was placed in an LSE Benchtop Shaking Incubator 222DS (Corning, Tewksbury, MA) at 25 °C for 12 days, with sampling every 3 days for chemical profiling of the beverage.

### Kombucha beverage flavoring: Secondary fermentation

After 12 days of fermentation, the SCOBY and the newly formed SCOBY were removed from the beverage by decanting. Three formulations were prepared in a randomized complete design, where all the formulations contained 250 mL of the kombucha beverage obtained in the first fermentation, plus formulation 1: 250 mL of pasteurized chote juice, formulation 2: 250 g of fruit pieces (85-87 % moisture) and formulation 3: 62.5 mL of pasteurized chote juice and 62.5 g of fruit pieces. For the secondary fermentation, the bottles were kept refrigerated at 4 °C for 4 days, with samples taken daily. The experiment was conducted in duplicate.

### Sensory evaluation of fermented flavored kombucha beverage

To determine the acceptability indexes of the kombucha beverage, a 7-point hedonic scale was used, considering the criteria of flavor, color, odor, and general acceptability. The evaluation was carried out in the Fruit and Vegetable Workshop of the Tecnológico Nacional de México/IT of Ciudad Valles with 30 untrained judges between 18 and 23 years of age. During the sensory evaluation, the three formulations were tasted, along with the traditional kombucha beverage (Formulation 4) and the pasteurized fruit juice (Formulation 5), which were used as controls for results comparison with the three formulations developed in this research.

### Chemical profile of the fermented flavored kombucha beverage

During the first and second fermentation, pH, titratable acidity, and dissolved solids (°Brix) were monitored. The pH was measured using a Hi98103 Hanna pH Checker potentiometer following Mexican Standard NMX-F-317-NORMEX-2013. Titratable acidity was measured according to Mexican Standard NMXFF-011-1982, using volumetry and expressing the result as a percentage of lactic acid and acetic acid (% w/V, g/100 mL juice). The soluble solids content was

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measured with a refractometer according to Mexican Standard NMX-F-436-SCFI-2011, with the result expressed as °Brix.

### Statistical Analysis

Sensory evaluation data were analyzed using a Tukey mean comparison test in STATISTICA version 7.0 software (Statsoft, Tulsa, OK, USA) to observe significant differences between the formulations developed and the controls. The chemical profile data were analyzed using averages, standard deviations, and coefficients of variation between replicates of the analytical methods.

## Results and Discussion

### Sensory evaluation

The analysis of the sensory evaluation data for the developed formulations and controls showed that there were no significant differences in the attributes of color, odor, and flavor according to Tukey's test ($\alpha$ = 0.050). These values ranged from "I dislike it moderately" to "I like it a little." However, significant differences were observed when analyzing the overall acceptability of the developed formulations and the controls. Formulation 3 presented the highest acceptability with a value of 4.6, considered as "I dislike it a little" (Table 1). This result indicates better overall acceptability compared to the preference for kombucha alone and pasteurized juice (4.6 vs. 3.80 and 3.71, respectively). These results are expected due to the chemical characteristics of the beverage, as it is rich in acids such as acetic acid, and the palate of untrained judges is not habituated to these flavors.

**Table 1. Results of sensory evaluation of the fermented kombucha beverage flavored with the *P. aculeata* fruit.**

| Formulation | Color | Odor | Flavor | General |
|---|---|---|---|---|
| 1 | 4.80 ± 1.30 $^{b}$ | 3.43 ± 1.77 $^{b}$ | 4.47 ± 1.93 $^{b}$ | 4.23 ± 1.45 $^{ab}$ |
| 2 | 5.03 ± 1.40 $^{b}$ | 3.60 ± 1.57 $^{b}$ | 3.93 ± 1.66 $^{b}$ | 4.19 ± 1.11 $^{ab}$ |
| 3 | 4.97 ± 1.27 $^{b}$ | 4.17 ± 1.56 $^{b}$ | 4.67 ± 1.67 $^{b}$ | 4.60 ± 1.20 $^{a}$ |
| 4 | 5.33 ± 1.63 $^{b}$ | 3.53 ± 1.70 $^{b}$ | 2.53 ± 1.74 $^{a}$ | 3.80 ± 0.87 $^{ab}$ |
| 5 | 3.07 ± 1.80 $^{a}$ | 3.87 ± 1.89 $^{b}$ | 4.20 ± 2.16 $^{b}$ | 3.71 ± 1.13 $^{b}$ |

Different letters are statistically different, according to Tukey's test ($\alpha$ = 0.05).

The sensory evaluation indicators are described in the spider charts (Figure 1), where the distribution of the hedonic scale, represented by the external numbers 1 (I dislike it very

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much) to 7 (I like it very much), and the relative frequency of acceptability, represented by the internal numbers, are observed. Chote juice (Formulation 5) showed a low acceptability index for color, odor, and taste. However, when the juice is incorporated into the kombucha, it improves its sensory qualities. Regarding color, the three formulations with chote juice, fruit and chote juice-fruit remained on the hedonic scale of 5 and 6. In color and flavor terms, Formulations 1 and 3 reached the best acceptability indexes of 5 and 6. When we compare these results with traditional kombucha, we observe that the chote addition reduces the acceptability level in color terms, whereas the flavor and odor indexes improved in Formulations 1 and 3.

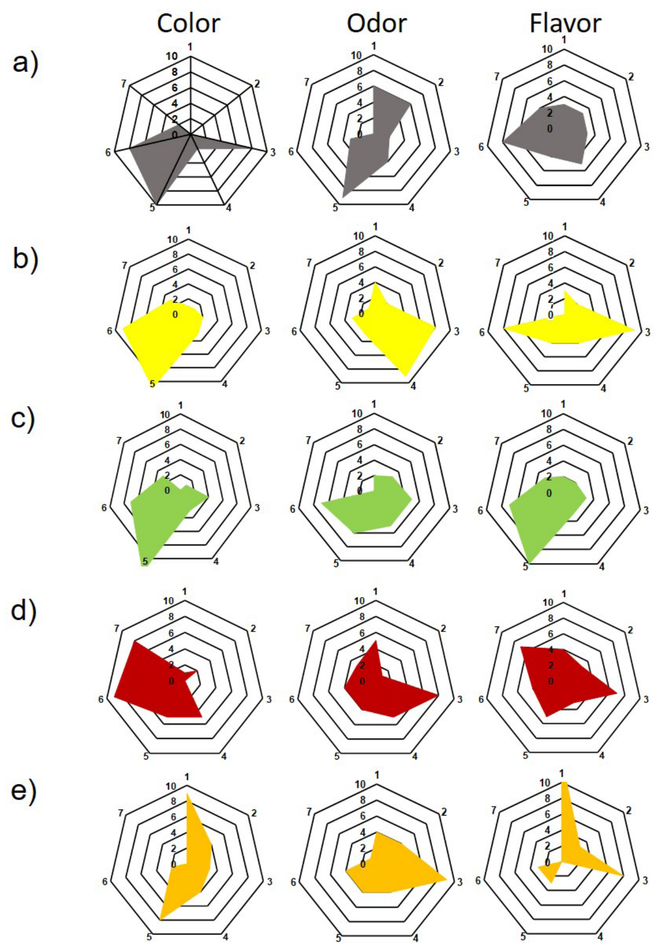


**Figure 1. Results of sensory evaluation of kombucha beverages flavored with *P. aculeata* fruit.**

a) Formulation 1 (kombucha + juice), b) Formulation 2 (kombucha + fruit), c) Formulation 3 (kombucha + juice + fruit), d) Formulation 4 (kombucha), and e) Formulation 5 (juice).

## Chemical profile of the fermented flavored kombucha beverage

During the 12-day fermentation period, it was observed that the kombucha beverage experienced a pH decrease from an initial value of 3.5 to a final value of 3.0 (Figure 2). These results are consistent with previous reports (Guzmán-Ortiz, 2021; Navarro, 2021; Tran *et al.,* 2020), which noted that the pH values of kombucha after 12 days typically range from 5.0 to 3.0. In the three formulations of kombucha flavored with the *P. aculeata* fruit, final pH values of 3.95 to 4.15 were obtained on the 4$^{th}$ day of fermentation, corresponding to the 2$^{nd}$ fermentation.

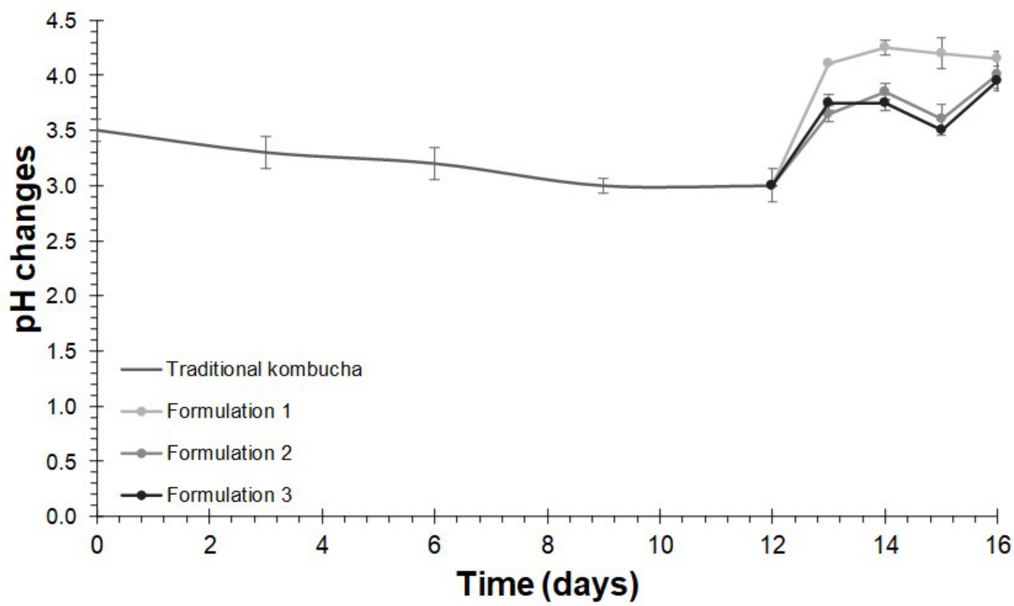


**Figure 2. pH changes during the first and second fermentation of kombucha fermented beverage production.**

Regarding titratable acidity (Figure 3), the obtained values in this research are 3.58 g/L of acetic acid in the first fermentation and 3.9-4.03 g/L in the three formulations obtained in the second fermentation (kombucha flavored with the *P. aculeata* fruit). This behavior may be due to the initial sucrose concentration, as other authors have reported titratable acidity values expressed as acetic acid of 5.6, 8.36, and 11 g/L when using different initial sucrose concentrations (70 and 100 g/L) and varying fermentation times of 15, 18, and 30 days (Leal *et al.,* 2018; Tran *et al.,* 2020). Acetic acid is important for the pH and unwanted microbial growth control (Martinez-Leal *et al.,* 2018). Under fermentation conditions the kombucha beverage with an initial sucrose concentration of

100 g/L and fermentation for 18 days, a lactic acid concentration of 0.18 g/L has been reported, which is lower than that found in the three formulations developed in this study, which fluctuates between 5.65-6.05 g/L in the kombucha beverage flavored with the *P. aculeata* fruit. Lactic acid is of utmost importance for the attribute of preventing constipation, as it stimulates the peristaltic movements of the intestine, contributing to adequate evacuations (Velázquez-López *et al.,* 2018). The literature indicates that the microorganisms responsible for the organic acids production during the beverage elaboration, which in turn contribute to pH changes of the beverage, are attributed to the presence of acetic acid bacteria, yeasts, and lactic acid bacteria (Tran *et al.,* 2020).

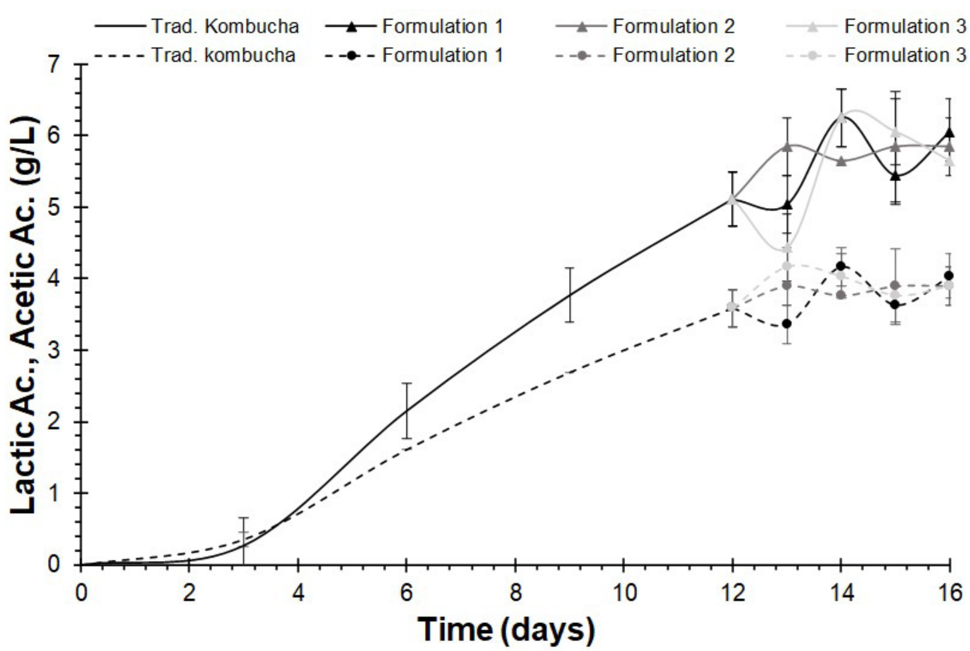


**Figure 3. Titratable acidity during the first and second fermentation of kombucha fermented beverage production.**

Lactic acid (-▲-) and acetic acid (-▲-).

The metabolism of the microorganisms present during kombucha beverage fermentation requires the consumption of substrates or sugars present in the beverage. Figure 4 illustrates the consumption of dissolved soluble solids measured by °Brix during the first fermentation. At this stage, based on obtained data, it can be suggested that there is dynamic growth of microorganisms, where they consume substrates and produce metabolites such as organic acids that contribute to a decrease in the pH of the medium. According to Tran *et al.* (2020), yeasts contribute by producing simple sugars and ethanol from the sucrose and polysaccharides degradation, while acetic acid bacteria consume glucose and ethanol to produce organic acids. In the second fermentation, an

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increase in dissolved soluble solids was observed as a consequence of adding *P. aculeata* fruit, with varying values reaching 4.5-5.0 °Brix by the end of 16 days. This gradual increase up to day 15 is associated with the release of soluble sugars from the polysaccharides contained in the fruit, facilitated by microbial activity. Some studies have indicated that sucrose is hydrolyzed to glucose and fructose by invertase produced by yeasts in the kombucha microbial consortium (Jafari *et al.,* 2020). Additionally, the fruit addition in the three formulations contributed to decreasing acidity in the beverage, and increasing pH. These effects caused by the fruit addition generated appealing sensory characteristics, improving the flavor profiles in formulations 1 and 3.

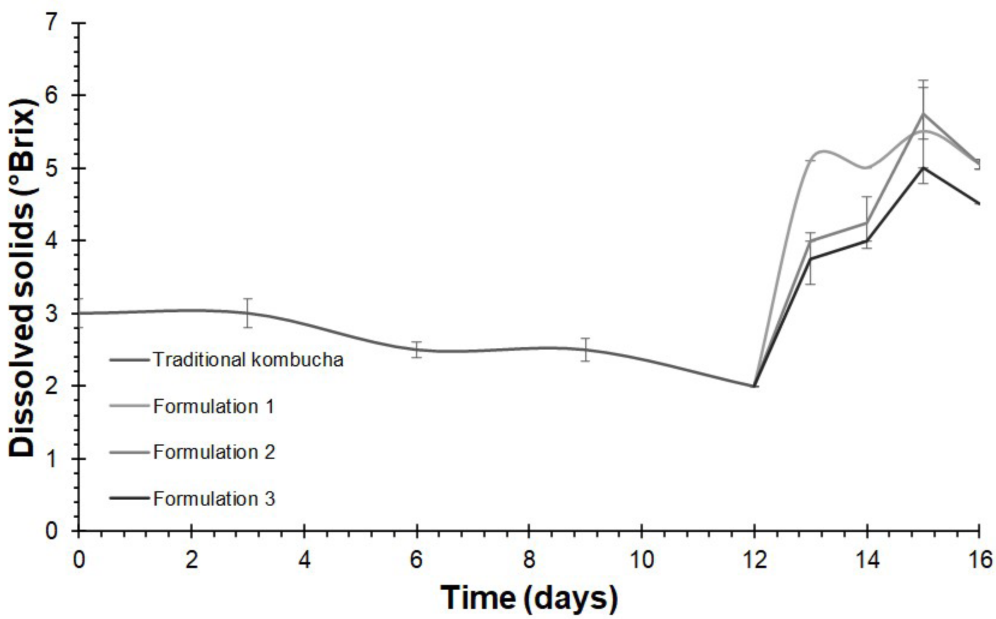


**Figure 4. Soluble solids content during the first and second fermentation of kombucha fermented beverage production.**

Several raw materials have been used for the first kombucha fermentation, such as coffee, apples, grapes, pears, carrots, and broccoli, among others (Anantachoke *et al.,* 2023; Júnior *et al.,* 2022). Additionally, different alternative substrates have been used for flavoring the kombucha beverage during secondary fermentation, including passion fruit, mango, tangerine, melon, and pineapple (Fernández Ormaza & Muñoz Jiménez, 2022; Luvison *et al.,* 2023; Stevens, 2019). These findings, combined with the results obtained in this study, reinforce that the *P. aculeata* fruit can serve as a low-cost alternative for flavoring in a kombucha-type beverage.

## Conclusions

This study represents the first report on the *P. aculeata* fruit inclusion as a low-cost alternative flavoring for a kombucha analog beverage. According to the obtained data, the chote addition to kombucha positively influences the overall beverage acceptance, with formulation 3, consisting of chote juice and fruit, being preferred. However, further studies are necessary to explore the biological activities of the beverage and its potential as a substrate from the beginning of the fermentation process for developing kombucha-type beverages.

## Author contribution

Work conceptualization, FVH; methodology development, JJGG, CSF; software management, FVH; experimental validation, JJGG, CSF, FVH, JLGE; analysis of results, JJGG, FVH, JLGE; data management, JJGG, FVH, JLGE; manuscript writing and preparation, FVH, JLGE; FVH, JLGE; project manager, FVH; fund acquisition, FVH. All authors of this manuscript have read and accepted the published version of the manuscript.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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