

Article

Development of Smoothies Fermented with Kombucha Microorganisms: Sensory Characteristics, Functional Properties, and Microbiological Aspects

Lorene Simioni Yassin ¹, Camila Gomes Sheleidres ¹ , Thaís Estéfane Fischer ¹,
Acácio Antonio Ferreira Zielinski ² , Paulo Ricardo Los ³, Luiz Gustavo Lacerda ^{1,3} , Aline Alberti ^{1,3} 
and Alessandro Nogueira ^{1,3,*} 

- ¹ Graduate Program in Food Science and Technology, State University of Ponta Grossa (UEPG), Av. Carlos Cavalcanti 4748, Ponta Grossa 84030-900, PR, Brazil; nutrilo@yahoo.com.br (L.S.Y.); camilagshleidres@hotmail.com (C.G.S.); tef.fischer@gmail.com (T.E.F.); luizgustavo75@gmail.com (L.G.L.); alinealberti@gmail.com (A.A.)
 - ² Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Campus Reitor João David Ferreira Lima, Florianópolis 88040-970, SC, Brazil; aczielinski@gmail.com
 - ³ Department of Food Engineering, State University of Ponta Grossa (UEPG), Av. Carlos Cavalcanti 4748, Ponta Grossa 84030-900, PR, Brazil; pauloricardolos@yahoo.com.br
- * Correspondence: alessandronog@yahoo.com.br

Abstract

Smoothies and kombucha are beverages appreciated by contemporary consumers due to their appealing flavor, convenience, and perceived health benefits. This study aimed to develop fruit- and white tea-based smoothies with high sensory and functional quality, and to evaluate the effects of fermentation with kombucha microorganisms. The smoothie base (70%) was composed of 60% strawberry pulp and 40% cryoconcentrated apple juice. Ten formulations were designed using a centroid simplex approach and subjected to sensory analysis. The two most accepted by consumers (E5: 15% blackberry pulp and 15% white tea; E10: 5% blackberry pulp and 5% acerola pulp, and 20% white tea) were fermented for 10, 15, and 20 h. The formulations were evaluated through sensory analysis. Fermentation led to significant ($p < 0.05$) reductions in glucose, fructose, and caffeine contents, while significantly ($p < 0.05$) increasing acidity, total phenolic content, ascorbic acid levels, and color intensity. Moreover, the fermented smoothie exhibited higher α -glucosidase inhibitory potential. One fermented smoothie (E5 fermented for 15 h) showed desirable sensory and functional properties. Therefore, this study demonstrates the successful development of smoothies, fermented or non-fermented with kombucha microorganisms, characterized by strong functional attributes and high sensory acceptance.

Keywords: functional beverage; sensory quality; technological innovation; Kombucha-Fermented Smoothies



Academic Editor: Spiros Paramithiotis

Received: 12 September 2025

Revised: 30 October 2025

Accepted: 5 November 2025

Published: 8 November 2025

Citation: Yassin, L.S.; Sheleidres, C.G.; Fischer, T.E.; Zielinski, A.A.F.; Los, P.R.; Lacerda, L.G.; Alberti, A.; Nogueira, A. Development of Smoothies Fermented with Kombucha Microorganisms: Sensory Characteristics, Functional Properties, and Microbiological Aspects.

Fermentation **2025**, *11*, 637.

<https://doi.org/10.3390/fermentation11110637>

Copyright: © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

(<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Smoothies are often considered “superfoods” due to their nutrient-rich composition, potentially associated with positive health effects [1]. They are prepared from fruit juices or pulps, often combined with cereals, vegetables, honey, tea, or milk, and provide bioactive compounds that occur naturally or can be enhanced through fermentation, thereby improving functionality [2]. Beyond basic nutrition, smoothies promote increased fruit and

vegetable intake, especially among younger populations [3] and contribute to the prevention of chronic diseases such as obesity, diabetes, and cardiovascular disorders [4]. These benefits are largely attributed to phenolics, anthocyanins, vitamins, minerals, and dietary fiber, which are often scarce in commonly consumed foods [5]. Furthermore, smoothies provide a suitable matrix for incorporating functional ingredients, such as probiotics or plant extracts, thereby reinforcing their potential as functional foods [1,3]. The growing consumer demand reflects this potential, as kombucha is part of the broader global functional food and beverage market, which is valued at USD 4.26 billion in 2024 and projected to reach USD 13.09 billion by 2033 [6].

Smoothies first appeared in the United States in the 1960s and regained popularity in the early 2000s, rapidly expanding worldwide with diverse formulations adapted to consumer preferences and technological advances. Their phytochemical composition and sensory attributes depend on raw materials and processing methods. However, despite their functional appeal, smoothies do not always achieve consumer acceptance. High production costs and limited sensory appeal may reduce consumption and market availability [2].

Kombucha, in turn, is a slightly acidic and sweet fermented tea produced from *Camellia sinensis* leaves and sugar using a symbiotic culture of acetic acid bacteria (*Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species), lactic acid bacteria (*Lactobacillus*, *Lactococcus*), and yeasts (*Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*) [7,8]. Therefore, kombucha fermentation involves three simultaneous processes—alcoholic, lactic, and acetic—driven by the coexistence of various yeasts and bacteria. These microorganisms establish a strong symbiotic relationship that inhibits the growth of contaminants. The process begins with osmotolerant species and is later dominated by acid-tolerant microorganisms [9]. During fermentation, certain acetic acid bacteria form a floating biofilm (scooby) on the surface of the medium. The main organic acids produced include acetic, gluconic, tartaric, malic, and, in smaller amounts, citric acid, which together contribute to the characteristic sour taste of kombucha [10].

Its consumption dates back to China in 220 BCE and later spread to Japan (414 CE) and other regions, where it became valued for its distinctive sensory attributes, and probiotic and prebiotic potential [8,11]. Although traditionally made from tea, kombucha has also been produced from herbal infusions, fruit juices, milk, and unconventional plants, yielding beverages with comparable sensory profiles and promising functional properties [12].

In this study, strawberry, blackberry, and acerola pulps, cryoconcentrated apple juice, and white tea were selected to formulate smoothies with demonstrated functional potential and high sensory acceptance. Additionally, fermentation with kombucha microorganisms was evaluated as a strategy to enhance their physicochemical properties, bioactive compound contents, antioxidant activity, and sensory attributes.

2. Materials and Methods

2.1. Material

The ingredients were obtained from local suppliers in Ponta Grossa, Brazil. Organic pulps of acerola (*Malpighia emarginata*), blackberry (*Rubus* spp.), and strawberry (*Fragaria* spp.) were purchased from Verde Brasil (Piraí do Sul, Paraná, BR) and stored at -15 to -18 °C until use in smoothie preparation. Cryoconcentrated Gala apple juice (*Malus domestica* Borkh.) was produced according to the methodology described by Carraro et al. [13] and standardized to 38 °Brix. White tea (*Camellia sinensis* (L.) Kuntze) (Leão Alimentos e Bebidas Ltda[®], Fazenda Rio Grande-PR, Brazil) was prepared by infusing 20.0 g of dry

leaves in 1 L of distilled water at 80 °C for 7.5 min in a covered Erlenmeyer flask under magnetic stirring, following the procedure described by Zielinski et al. [14].

The Symbiotic Culture of Bacteria and Yeast (SCOBY) used for kombucha development was obtained in its hydrated form from a certified source (The Kombucha Hub[®], White Labs Laboratory, Frankfurt, Germany). According to the manufacturer, the cellulose plate contained the following bacterial strains: *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus aerophilus*, *Bacillus safensis*, *Bacillus altitudinis*, *Bacillus aerophilus*, *Bacillus aryabhattai*, *Bacillus subtilis*, *Acetobacter tropicalis*, *Gluconacetobacter saccharivorans*, *Micrococcus* sp., *Gluconacetobacter rhaeticus*, *Paenibacillus taichungensis*, and yeasts: *Brettanomyces bruxellensis*, *Saccharomyces cerevisiae*, *Zygosaccharomyces* sp.

Lauryl sulfate tryptose (LST) broth (7142A, Acumedia[®], Neogen[®], Lansing, MI, USA) was used to detect thermotolerant coliforms and *Escherichia coli*. Total fungi and yeasts were enumerated on potato dextrose agar (K25–1022, Kasvi, Pinhais, PR, Brazil) acidified with tartaric acid, and lactic acid bacteria were enumerated on MRS agar (Kasvi, Pinhais, PR, Brazil).

Folin–Ciocalteu reagent, standard Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (97% pure), TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-2-picrylhydrazyl), and the phenolic compound standards used for HPLC analysis (gallic acid (≥99%), chlorogenic acid (95%), catechin (98%), epigallocatechin (≥95%), epigallocatechin gallate (>95%), epicatechin gallate (≥98%), procyanidin B1 (98%), phloridzin (99%), and caffeine (>99%)) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol (>99.8%), Acetic acid (≥99.7%), and acetonitrile (99.9%) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Standards for sugar analysis were as follows: D-glucose and sucrose (99%) (Sigma-Aldrich, Steinheim, Germany) and fructose and glycerol (99%) (Merck, Darmstadt, Germany). Acetone (Sigma-Aldrich, St. Louis, MO, USA) and petroleum ether (Anidrol, Diadema, SP, Brazil) were used for carotenoid analysis. Ultrapure water (type 1) was used (Millipore, SP, Brazil). The culture media and reagents used were of analytical grade.

2.2. Methods

2.2.1. Blend Design for Smoothie Preparation

The proportions of the ingredients were determined using a simplex centroid mixture design implemented in Statistica software v.13.3 [15]. For the design, the sum of all ingredient proportions was constrained to 100%, while minimum and maximum limits for each component were established based on prior experimental results. This approach generated a total of 10 mixtures (E1 to E10) according to the experimental design, and the resulting models were subjected to statistical analysis. The beverage base was fixed at 70%, consisting of 60% strawberry pulp and 40% cryoconcentrated Gala apple juice. The mixture design for the remaining ingredients is presented in Table 1.

To prepare the smoothies, all ingredients were homogenized (~3 min) using a mixer (Black & Decker, M100, Uberaba, MG, Brazil). The blends were then transferred into glass bottles that had been previously sterilized in an autoclave (Phoenix[®] VA 13811, Phoenix Luferco, Araraquara, SP, Brazil) at 121 °C and 1.01 kgf/cm² for 15 min. The mixtures were stored under refrigeration (6–10 °C), and sensory analyses were performed on the same day of preparation. Aliquots of each blend were frozen for subsequent physicochemical and functional analysis.

Table 1. Experimental design using simplex centroid mixture planning for the development of smoothie formulations.

Assay	Factors (%)		
	Blackberry Pulp	Acerola Pulp	White Tea
E1	30 (1)	0 (0)	0 (0)
E2	0 (0)	30 (1)	0 (0)
E3	0 (0)	0 (0)	30 (1)
E4	15 (0.5)	15 (0.5)	0 (0)
E5	15 (0.5)	0 (0)	15 (0.5)
E6	0 (0)	15 (0.5)	15 (0.5)
E7	10 (0.33)	10 (0.33)	10 (0.33)
E8	20 (0.66)	5 (0.17)	5 (0.17)
E9	5 (0.17)	20 (0.66)	5 (0.17)
E10	5 (0.17)	5 (0.17)	20 (0.66)

Note: beverage base 70% (60% strawberry pulp and 40% cryoconcentrated Gala apple juice).

2.2.2. Sensory Analysis of Formulated Smoothies

Flash Profile analysis was performed on the smoothies according to the methodology proposed by Dairou and Sieffermann [16], using nine trained panelists (professors and students from the Food Engineering Program at the State University of Ponta Grossa), who were selected through an individual questionnaire, based on their experience with sensory analysis and descriptive methods. The study protocol was approved by the Ethics Committee of the State University of Ponta Grossa, Paraná, Brazil (CAAE n°. 56282916.0.0000.0105; approval n°. 1.649.931). Tests were conducted in individual booths under white light, and samples (50 mL) were served at 10–12 °C in triplicate, coded with three random digits. Data were analyzed using the Friedman test to assess differences among samples, followed by Generalized Procrustes Analysis (GPA) with Idiogrid[®] software (version 2.4) to obtain the consensus configuration, evaluate discrimination and repeatability, and generate descriptive maps.

2.2.3. Fermentation of Selected Smoothie Mixtures

The white tea infusion was filtered through Whatman n°. 1 paper. The filtered tea was transferred to 5 L glass fermenters. For kombucha fermentation, 100 g/L of sucrose was added to the tea. Once the mixture had cooled to 25 °C, a SCOBY (2.5% *w/v*) and 10% (*v/v*) of previously fermented kombucha were inoculated. The fermenter was covered with porous polyester fabric to ensure aerobic fermentation and incubated at varying temperatures for 7 days (4.5 pH). After fermentation, the SCOBY was removed, homogenized, and incorporated according to the proportions of white tea established in the experimental design: 15% for sample E5 and 20% for sample E10. The mixtures were incubated at 25 °C (Incubator MA 1415/300, Marconi, São Paulo, SP, Brazil) for 10, 15, and 20 h, resulting in samples E5F10, E10F10, E5F15, E10F15, E5F20, and E10F20.

To facilitate direct comparison between pre- and post-fermentation samples, all fermented samples were subjected to a second sensory evaluation together with the previously identified unfermented mixtures that performed best in the initial analysis. Only the samples achieving the highest scores in this second sensory evaluation were selected for subsequent analyses. These selected samples were stored frozen at −15 °C to −18 °C until analysis.

2.3. Physicochemical, Functional, and Microbiological Analyses

2.3.1. Physicochemical Analysis

Soluble solids were measured using a refractometer (ABBE AR 1000 S, Megabrix, São Paulo, SP, Brazil). The pH was determined using a calibrated pH meter (Tec-3 MP, Tecnal, São Paulo, SP, Brazil). Total titratable acidity was expressed as citric acid (g/100 mL). For titration, 10 g of smoothies was homogenized with 90 mL of distilled water and titrated with NaOH to pH 8.3, following AOAC [17].

2.3.2. Sugar Profile

The determination of glucose, fructose, and sucrose content was performed as described by Santos et al. [18] in a high-performance liquid chromatography (HPLC) system (Waters Alliance 2695, Waters Corporation, Milford, MA, USA) coupled with a refractive index detector (Waters RI 2414, Milford MA, USA) and a Sugar Pak™ column (300 × 6.5 mm i.d., Waters RI 2414, Waters Corporation, Milford MA, USA). Elution was performed at 30 °C with a flow rate of 0.5 mL/min using 5 mM sulfuric acid as the mobile phase. The areas of the peaks were determined and compared with the standard curves (sucrose, D-glucose, and D-fructose). Sugar was expressed as grams per 100 mL of smoothie.

2.3.3. Fiber Analysis

Total dietary fiber was determined gravimetrically using an enzymatic kit (Megazyme International, Bray, Co., Wicklow, Ireland), as described by IAL [19]. Results were expressed in g/100 mL.

2.3.4. Color Parameters

The smoothie colors were expressed in the Commission Internationale de l'Eclairage (CIE) L*, a*, and b* color space coordinates. The samples were analyzed using a digital colorimeter (CM-5-ID, Konica Minolta, Osaka, Japan) with Illuminant D65 and SpectraMAGIC NX (Color data software CM-S100w, v. 2.6, Osaka, Japan). Colors were obtained by converting CIELAB data (L*, a*, and b*) to other systems such as RGB, HEX (hexadecimal), and CYMK using the NIX® color sensor software (v. 3.0, Osaka, Japan). The chroma parameter (C*) related to color intensity was calculated (1), and the hue angle parameter (h°) was related to tonality (2) or (3):

$$C^* = ((a^*)^2 + (b^*)^2)^{1/2} \quad (1)$$

$$h^\circ = \tan^{-1} (b^*/a^*) + 180^\circ, \text{ when } a^* < 0 \quad (2)$$

$$h^\circ = \tan^{-1} (b^*/a^*), \text{ when } a^* > 0 \quad (3)$$

2.3.5. Apparent Viscosity

Apparent viscosity (mPa·s) was measured using a Brookfield RV DV-II + Pro rotary viscometer with SC4-21 spindle and SC4-13RPY adapter, and MVS1Y Flag Impeller in a TC-112P bath (Brookfield Engineering Labs, Middleboro, MA, USA). Ten grams of the sample were analyzed at 10 ± 0.5 °C for 2 min at 423× g (Centrifuge Excelsa II, Fanem, São Paulo, SP, Brazil). Viscosity was calculated using Rheocalc® software v. 3.2.

2.3.6. Determination of Individual Phenolic Compounds and Caffeine

Phenolic compounds and caffeine were analyzed by HPLC as previously described by Alberti et al. [20] and Yassin et al. [2]. The HPLC apparatus was a 2695 Alliance (Waters, Milford, MA, USA) equipped with a quaternary pump, auto-sampler, photodiode array detector PDA 2998 (Waters, Milford, MA, USA), and Symmetry C18 (4.6 × 150 mm,

3.5 mm) column (Waters). The samples (10 g) were lyophilized (LD 1500, Terroni, São Paulo, SP, Brazil) and solubilized in a solution of acetic acid (2.5%) and methanol (99.9%) (1:3 mL/mL). The samples were subsequently homogenized, centrifuged ($8160 \times g$, 20 min at 4 °C) (HIMAC CR-GII, Hitachi, Tokyo, Japan), filtered through a 0.22 mm nylon syringe filter (Waters, Milford, MA, USA), and stored in vials for analysis. The runs were monitored at 273 nm (caffeine), 280 nm (flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids), and 350 nm (flavonols). The identification and quantification of phenolic compounds were performed by comparing their retention time with that of standards and by using analytical curves.

2.3.7. Carotenoids

β -Carotene and lycopene were extracted and quantified according to Rodriguez-Amaya [21], modified by Zielinski et al. [22]. Briefly, 15 g of the sample was stirred with 30 mL of acetone at 200 rpm for 1 h. The acetone phase was removed via vacuum filtration, and pigments were transferred to petroleum ether, washed until complete acetone removal, and adjusted to 50 mL. Absorbances were measured at 450–470 nm, in triplicate, and results expressed as mg/100 g.

2.3.8. Total Phenolic Compounds, Ascorbic Acid, and Antioxidant Activity

Total phenols were quantified by the Folin–Ciocalteu method [23]. Ascorbic acid (AA) was measured according to Isabelle et al. [24]. TPC was corrected for AA content (4):

$$\text{Correct TPC (mg CQAE/L)} = (\text{TPC}) - (\text{AA}) \quad (4)$$

Antioxidant activity was determined using DPPH [25], ABTS [26], FRAP [27], and CUPRAC [28] methods, with Trolox standard curves. All assays were performed in quadruplicate using a microplate reader (Epoch, Synergy-BioTek, Winooski, VT, USA).

2.3.9. Microbiological Analysis

Microbiological analyses for the enumeration of lactic acid bacteria, thermotolerant coliforms, *Escherichia coli*, and total yeasts/molds, and the detection of the presence or absence of *Salmonella* spp., were performed according to Silva et al. [29]. Sterilized 0.1% (*w/v*) peptone saline was used as a diluent for microbial enumeration. Microorganism counts were carried out in triplicate, and the average value was expressed in UFC per mL.

2.3.10. α -Glucosidase Inhibition

Inhibitory activity was determined using the chromogenic method of McCue et al. [30]. Absorbance was measured at 405 nm, and inhibition was calculated as (5):

$$\% \text{ inhibition} = [(\text{Control Abs} - \text{Sample Abs}) / \text{Control Abs}] \times 100 \quad (5)$$

2.3.11. Statistical Analysis

Data were presented as mean and standard deviation. The homogeneity of variance was tested by Levene's test or F-test ($p \geq 0.05$). Differences between samples were evaluated using Student's *t*-test or one-way ANOVA, followed by Fisher's LSD test. Statistical analysis was performed using Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

The sensory evaluation of the ten formulations revealed significant differences among samples, as determined by Fisher's LSD test ($p < 0.05$). As shown in Figure 1A, the unfermented formulations E5 and E10 were the most preferred by the trained panelists.

Coded Sample	Σ of Orders
E1	152 ^{bc}
E2	207 ^e
E3	190 ^d
E4	135 ^b
E5	110 ^a
E6	146 ^{bc}
E7	147 ^{bc}
E8	138 ^b
E9	146 ^{bc}
E10	114 ^a

(A)

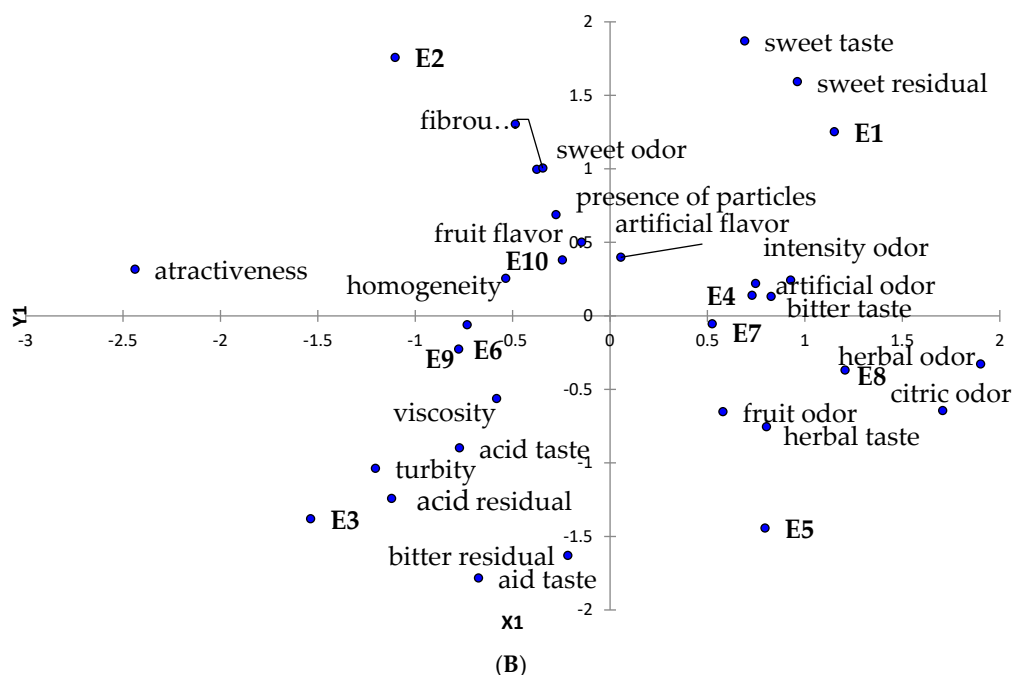


Figure 1. Preference of pre-fermentation smoothies (A) and GPA of tested mixtures (B). Note: Beverage base 70% (60% strawberry pulp and 40% cryoconcentrated Gala apple juice); E1: 30% Blackberry Pulp; E2: 30% Acerola Pulp; E3: 30% White Tea; E4: 15% Blackberry Pulp + 15% Acerola Pulp; E5: 15% Blackberry Pulp + 15% White Tea; E6: 15% Acerola Pulp + 15% White Tea; E7: 10% Blackberry Pulp + 10% Acerola Pulp + 10% White Tea; E8: 20% Blackberry Pulp + 5% Acerola Pulp + 5% White Tea; E9: 5% Blackberry Pulp + 20% Acerola Pulp + 5% White Tea; E10: 5% Blackberry Pulp + 5% Acerola Pulp + 20% White Tea; fibrou: fibrous residual. ^{abcde}: Different letters in the same column indicate a significant difference between the samples at a 5% significance level according to Friedman test.

Sample E5 (70% base, 15% blackberry pulp, and 15% white tea) was characterized by evaluators as having an herbal flavor with fruity, citrus, and herbal notes in the aroma (Figure 1). This formulation achieved the highest sensory scores, indicating a well-balanced flavor profile suitable for consumption, while also presenting relevant nutritional and functional attributes. The second preferred formulation (Figure 1A) was sample E10 (70% base, 5% blackberry pulp, 5% acerola pulp, and 20% white tea), which was described as fruity in flavor, with a sweet aroma, good homogeneity, slight fibrous residue, presence of small particles, and overall attractiveness (Figure 1B).

Although samples E2, E7, and E8 exhibited sensory attributes like those of E5 and E10, they received lower acceptance scores. This result may be attributed to differences in ingredient proportions, which either enhanced undesirable characteristics or masked the desirable attributes present in the formulations.

As the aim of this study was to develop smoothies with pronounced functional properties while maintaining good sensory acceptance, only samples E5 and E10, which were preferred by the panelists ($p > 0.05$) and showed statistically significant differences in preference, were selected for fermentation. These formulations were fermented for 10, 15, and 20 h at 25 °C and subsequently subjected to a new sensory evaluation.

The preference analysis (Figure 2A) indicated higher acceptance for samples E10, E5F15, and E5, suggesting that fermentation generally reduced palatability. This decrease in acceptance may be attributed to the loss of the typical smoothie profile, with the emergence of sensory descriptors resembling alcoholic beverages, particularly liqueurs, which became more evident at longer fermentation times. These results agree with the physico-chemical findings (Table 2). In addition, sensory responses appear to have been influenced

by panelists' prior expectations, since previous experiences and conceptual associations with smoothies are known to affect product perception [31]. Since the post-fermentation smoothies (unfamiliar samples) exhibited characteristics distinct from the unfermented formulations, the panel considered it necessary to exclude some descriptors and introduce new ones in the evaluation form, as shown in Figure 2. In this case, changes were observed in viscosity and acidity, as well as in the odor and flavor of both vinegar and alcohol.

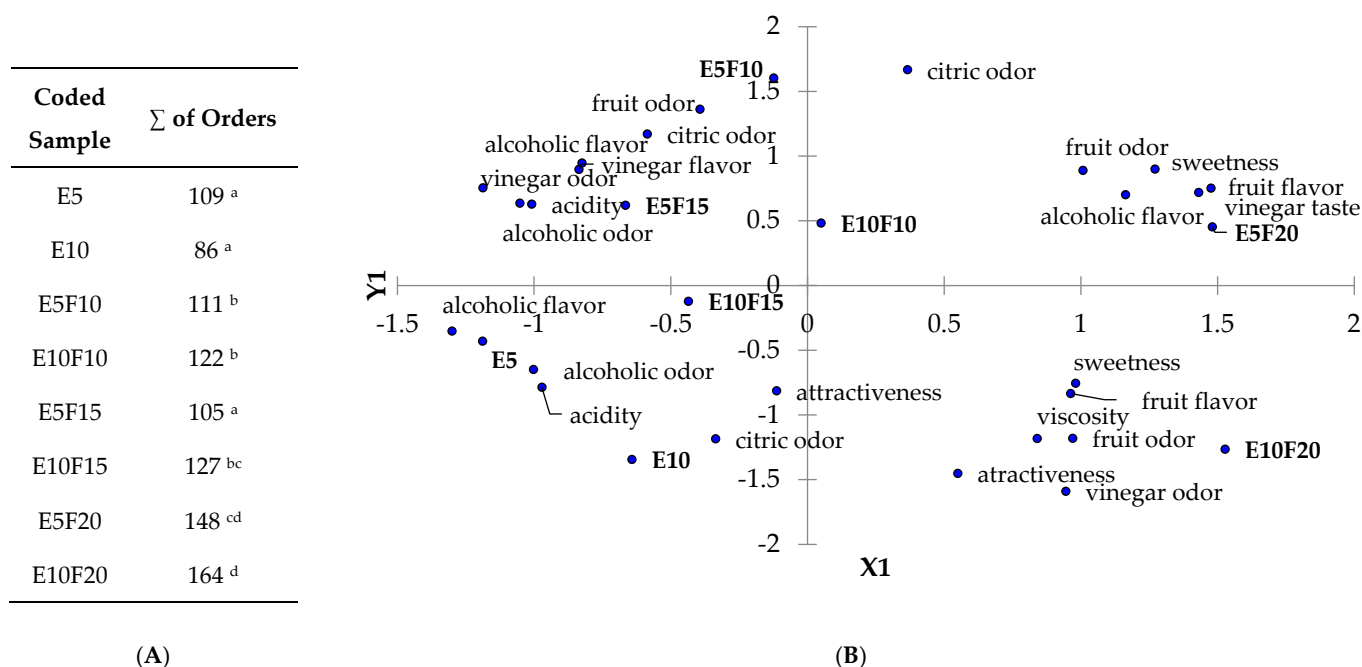


Figure 2. Preference (A) and GPA-based sensory analysis (B) of pre- and post-fermentation smoothies. Note: E5: experiment 5, E10: experiment 10, E5F10: experiment 5 fermented 10 h, E5F15: experiment 5 fermented 15 h, E5F20: experiment 5 fermented 20 h, E10F10: experiment 10 fermented 10 h, E10F15: experiment 10 fermented 15 h, E10F20: experiment 10 fermented 20 h. ^{abcd}: Different letters in the same column indicate a significant difference between the samples at a 5% significance level according to Friedman test. The lower grades correspond to the preferred samples due to the ordering methodology, in which the preferred one receives the number 1.

The lowest pH values were observed in the fermented smoothies, which can be attributed to the addition of kombucha. This beverage is characterized by the presence of organic acids like acetic, gluconic, glucuronic, lactic, malic, citric, and succinic acids, as well as other metabolites formed during fermentation, which may contribute to pH reduction [32].

For the most preferred fermented formulation (E5F15), a decrease in soluble solids ($^{\circ}$ Brix) and a 27.7% increase in total acidity were observed compared to its unfermented counterpart (E5), indicating a positive effect of fermentation on balancing the sweet and sour taste in the fermented smoothie. Additionally, fermentation reduced glucose and fructose contents by 13.33% and 16.11%, respectively. The greater reduction in fructose during fermentation may suggest a preference for fructose by the microorganisms in the scoby. Similar behavior has been reported by Sievers et al. [33] for the fermentation of London tea. Furthermore, the authors also noted different substrate consumption behaviors among various microorganisms, with isolated *Zygosaccharomyces* cells fermenting fructose at a higher rate, while *Acetobacter xylinum* converted glucose before fructose. In the study by Kallel et al. [34], glucose was preferred over fructose as a carbon source by kombucha microorganisms. This suggests that the composition of microorganisms in the scoby and

starter influences the fermentation process. Additionally, the fructose content may increase due to significant isomerization of glucose to fructose during fermentation [34].

Table 2. Physical–chemical and functional composition of selected smoothie formulations before (E5 and E10) and after (E5F15) kombucha fermentation.

Analytical Parameters	Selected Assays		
	E5	E5F15	E10
Soluble solids (°Brix)	5.17 ^b ± 0.15	4.92 ^c ± 0.15	7.08 ^a ± 0.15
Glucose (g/100 mL)	1.80 ^b ± 0.06	1.56 ^c ± 0.00	2.75 ^a ± 0.04
Fructose (g/100 mL)	2.98 ^b ± 0.06	2.50 ^c ± 0.00	4.31 ^a ± 0.00
Sucrose (g/100 mL)	0.30 ^a ± 0.00	0.31 ^a ± 0.05	0.15 ^b ± 0.04
Fibers (g/100 mL)	0.50 ^a ± 0.01	0.42 ^c ± 0.01	0.46 ^b ± 0.02
pH	3.56 ^b ± 0.01	3.52 ^c ± 0.01	3.59 ^a ± 0.01
Acidity (g/100 mL)	0.650 ^b ± 0.001	0.830 ^a ± 0.015	0.68 ^b ± 0.03
Viscosity (mPa·s)	39.68 ^b ± 0.01	12.33 ^c ± 0.01	59.83 ^a ± 0.01
Chroma	41.35 ^c ± 0.06	49.30 ^b ± 0.13	74.60 ^a ± 0.03
Hue angle (°)	21.37 ^c ± 0.17	27.07 ^b ± 0.04	44.02 ^a ± 0.02
Total phenols (mg CTE/L)	10,758 ^b ± 92	11,641 ^a ± 152	10,377 ^c ± 99
β-carotene (mg/L)	9.68 ^a ± 0.06	10.60 ^a ± 0.04	8.47 ^a ± 0.13
Lycopene (mg/L)	69.62 ^a ± 0.06	60.77 ^a ± 0.23	61.45 ^a ± 0.16
Ascorbic acid (mg/L)	5.40 ^c ± 0.02	14.60 ^b ± 0.09	17.02 ^a ± 0.02
ABTS (μmol TE/L)	10,390 ^{ac} ± 110	8568 ^b ± 135	11,084 ^a ± 98
CUPRAC (μmol TE/L)	12,462 ^b ± 151	10,222 ^e ± 140	16,396 ^a ± 80
DPPH (μmol TE/L)	39,395 ^b ± 187	38,008 ^c ± 61	44,800 ^a ± 92
FRAP (μmol TE/L)	12,462 ^b ± 151	10,222 ^e ± 140	16,316 ^a ± 211
Gallic acid (mg/L)	21.20 ^b ± 0.31	nd	nd
Catechin (mg/L)	98.97 ^a ± 3.23	26.96 ^c ± 0.20	78.18 ^b ± 0.35
Epicatechin (mg/L)	14.24 ^b ± 0.51	29.80 ^a ± 0.94	nd
Epigallocatechin (mg/L)	nd	nd	nd
Epigallocatechin gallate (mg/L)	nd	nd	nd
Epicatechin gallate (mg/L)	nd	nd	nd
Phloridzin (mg/L)	2.92 ^b ± 0.06	4.17 ^a ± 0.06	0.23 ^c ± 0.01
Chlorogenic acid (mg/L)	40.92 ^b ± 0.41	43.96 ^a ± 0.08	34.81 ^c ± 0.11
Procynidin B1 (mg/L)	64.76 ^a ± 1.59	nd	31.94 ^b ± 0.82
Caffeine (mg/L)	337.53 ^d ± 1.70	101.56 ^e ± 1.35	407.46 ^c ± 1.19

Note: E5: 70% base, 15% blackberry pulp, and 15% white tea; E10: 70% base, 5% blackberry pulp, 5% acerola pulp, and 20% white tea; E5F15: experiment 5 after 15 h of fermentation. °Brix: numerical scale that measures the amount of soluble solids. CTE: catechin equivalent. TE: Trolox equivalent. mPa.s: milli Pascal per second. ^{abcde}: Different letters in the same line indicate a significant difference between the samples at a 5% significance level according to Fisher's LSD test. nd: not detected.

Kombucha itself contained higher sugar concentrations than the other formulations, with 3.88 ± 0.04 g/100 g of sucrose, 1.84 ± 0.14 g/100 g of glucose, and 2.06 ± 0.01 g/100 g of fructose. Sucrose levels were approximately 12.9 times higher than those observed in the fruit-based smoothies (Table 2). This is due to the addition of sucrose as the primary fermentation substrate. During the initial stages of kombucha fermentation, sucrose is the first carbon source metabolized by acetic acid bacteria and yeasts. In the first three days, sugar levels reach their maximum concentration, after which they decrease, while acid production increases, and the pH must be carefully monitored to remain between 4 and 5, depending on culture origin and fermentation conditions [35,36]. In the E5 and E10 smoothie formulations, kombucha was employed as an inoculum at 15% and 20%, respectively, replacing white tea.

The fruit pulps used in the formulations contributed relatively low sugar concentrations. Strawberries, the major fruit component, contain approximately 9 g total sugars/100 g

pulp, while acerola provides up to 6 g/100 g and blackberries up to 7 g/100 g. Apple cryoconcentrate, used in the base, contributed the most to the sugar profile, with about 38 g sugars/100 g, highlighting its strong influence on the final composition.

Fermentation also significantly affected viscosity, which decreased by 3.3-fold compared to the non-fermented smoothies, directly influencing sensory preference. Viscosity is an important quality attribute in smoothies, contributing to sensory perception and consumer appreciation, even though no standard has been established for this parameter [37]. The reduction in viscosity is likely associated with the production of hydrolytic enzymes by kombucha-associated microorganisms [10].

Pratama et al. [38] observed a significant reduction in crude fiber content following the addition of different doses of kombucha to fermented fish silage, possibly due to the enzymatic activity of microorganisms present in kombucha, particularly *Lactobacillus* sp. and *Saccharomyces* sp., which can degrade crude fiber into simpler carbohydrates.

In contrast, a positive effect of fermentation was observed on color attributes. Chroma (C) values indicated that fermentation enhanced color saturation, resulting in a more vivid and intense appearance compared to the unfermented control, though still less intense than the E10 formulation, which included acerola pulp. The Hue angle confirmed differences in tonality, with acerola contributing additional red and yellow pigments, shifting the overall color perception [39].

Strawberries, the predominant fruit, are known for their high anthocyanin content, particularly pelargonidin derivatives, which strongly influence color but are highly sensitive to processing, such as fermentation [40]. Blackberries also contributed to the red hue due to the presence of cyanidin-3-glucoside, a pigment with strong antioxidant potential [41]. In the case of E10, the inclusion of acerola resulted in a hue angle of 44°, corresponding to a red–yellow tonality that distinguished it from the other formulations.

Fermentation of sample E5F15 under the experimental conditions significantly ($p < 0.05$) increased the total phenolic content by 8.2% compared with its unfermented counterpart. This effect can be attributed to the metabolic activity of kombucha microorganisms, which promote the degradation of complex phenolic compounds into simpler forms, thereby enhancing the measurable phenolic content in the beverage [10]. Probably, these newly formed phenolic compounds, or those released solely by enzymatic action, have low antioxidant activity.

Previous studies have also reported an increase in ascorbic acid content after tea fermentation, as microorganisms can produce this compound during the process [9,10]. In the present study, no ascorbic acid was detected, whereas fermentation increased the concentration of ascorbic acid to 11.53 mg/L. This indicates the positive effect of modifying the bioactive profile through the fermentation of white tea by kombucha microorganisms. Additionally, fermentation increased the ascorbic acid concentration by 2.7-fold compared to the unfermented sample. However, its level remained lower than that observed in the unfermented E10 formulation, most likely due to the presence of acerola pulp in E10, which naturally provides high amounts of ascorbic acid and was absent in both E5 and E5F15 (Table 2).

Fermentation did not enhance the antioxidant activity of the tested formulations. Among the samples, E10 consistently showed the highest antioxidant capacity across the four assays (ABTS, CUPRAC, DPPH, and FRAP). This effect can be partly explained by its higher ascorbic acid content, which was 14.21% greater than that of E5F15, in addition to its phenolic profile. Compounds such as catechin, phloridzin, chlorogenic acid, and procyanidins (Table 2), likely derived from the fruit blend—particularly apple in the base formulation and acerola pulp—appear to have contributed synergistically to the antioxidant potential of E10.

When ingredients were analyzed individually, white tea exhibited superior antioxidant activity compared with kombucha across all methods applied (White tea: ABTS = 10,992; CUPRAC = 12,022; DPPH = 35,217; FRAP = 12,022; Kombucha: ABTS = 9312; CUPRAC = 10,689; DPPH = 32,337; FRAP = 10,689; all values expressed as $\mu\text{mol TE/L}$). The white tea used in this study contained mainly epigallocatechin gallate (EGCG) and other phenolic compounds such as gallic acid, 1,2,6-trigalloylglucose, and caffeine, consistent with its minimal processing compared to green and black teas [42]. This compositional difference may explain the lower antioxidant activity of kombucha prepared with white tea, unlike reports for green- and black-tea-based kombuchas. In green tea, catechins—particularly EGCG—represent 30–50% of total solids and up to 90% of flavonoids, whereas in black tea, catechins are polymerized into theaflavins and thearubigins during fermentation [43].

In the present study, kombucha prepared with white tea initially contained epigallocatechin and EGCG. However, their concentration decreased by 71.66% and 34.43%, respectively, after fermentation. Gallic acid and epicatechin gallate, which were present in white tea before fermentation, were not detected in the fermented kombucha, suggesting their biotransformation into compounds with lower antioxidant activity. Consequently, the fermented smoothie (E5F15) exhibited reduced antioxidant activity compared with its unfermented counterpart (E5), although certain compounds increased in concentration: epicatechin (2-fold), phloridzin (1.5-fold), and chlorogenic acid (+7%).

These findings highlight a gap in current knowledge regarding the impact of fermentation on white tea, as most studies have focused on green or black teas, which often exhibit enhanced antioxidant activity after approximately seven days of fermentation [44]. Another relevant observation concerns the caffeine profile: white tea contained three times more caffeine than its kombucha derivative (2097 vs. 684 mg/L). The fermented sample (E5F15) had 235.97 mg/L less caffeine than the unfermented E5, consistent with the role of methylxanthines as substrates for microbial metabolism, particularly in bacterial cellulose production [7]. Carotenoid content did not differ significantly among samples, and as expected, these pigments were not detected in kombucha or tea.

Table 3 presents the microbiological analysis of the developed smoothies. Regarding total coliforms, all formulations complied with the limits established for juices. Notably, the formulation E5F15 showed a standard plate count six times higher, and mold and yeast count approximately 250,000 times higher than those of the unfermented control, a result consistent with the expected microbial profile of kombucha. This beverage is typically associated with the presence of acetic acid bacteria, such as *Bacterium gluconicum*, *Acetobacter aceti*, and *Acetobacter pasteurianus*, as well as yeasts including *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Zygosaccharomyces rouxii* [8]. Although none of these microorganisms are currently classified as probiotic strains by regulatory agencies, several studies have highlighted their biotechnological potential. Recent research has particularly emphasized the optimization of fermentation conditions to enhance the synthesis of metabolites with potential functional effects in foods and beverages, such as glucuronic acid and D-saccharic acid-1,4-lactone. These compounds are regarded as key contributors to the prophylactic properties attributed to kombucha [36].

Traditionally, dairy products and their derivatives have been the primary food sources of probiotics. However, due to increasing concerns related to allergies and intolerances that restrict dairy consumption, fruit- and vegetable-based beverages are emerging as promising alternative matrices for delivering probiotics. Their refreshing character and widely appreciated flavor may further encourage consumer acceptance. One of the main challenges for these non-dairy fermented products, as also observed in dairy matrices, is ensuring the viability of probiotic microorganisms throughout processing and storage. Nevertheless, the

technological and functional attributes of these novel products may represent a competitive advantage in the development and marketing of functional beverages [11].

Table 3. Microbiological analysis of developed smoothies of selected smoothie formulations before and after kombucha fermentation.

Sample Code	Total Coliforms 45 °C (NMP/mL)	Standard Count (UFC/mL)	Molds and Yeasts (UFC/mL)
E5	<3.0 ^a	1.0×10^4 ^b	1.0×10^2 ^c
E5F15	<3.0 ^a	6.0×10^4 ^a	2.55×10^6 ^a
E10	<3.0 ^a	1.0×10^3 ^c	4.0×10^2 ^b

Note: E5: experiment 5, E10: experiment 10, E5F15: experiment 5 after 15 h of fermentation. ^{abc}: Different letters in the same column indicate a significant difference between the samples at a 5% significance level according to Fisher's LSD test.

Regarding α -glucosidase inhibitory activity, which slows down the digestion and absorption of glucose from the diet, reducing postprandial hyperglycemia (blood sugar spikes after eating), Table 4 indicates that the unfermented formulation (E5) and kombucha showed similar results. In contrast, the fermented smoothie (E5F15) exhibited 14.6% greater inhibitory activity compared to both the unfermented formulation (E5) and kombucha, and an 18.5% higher activity than the E10 formulation. Given that the IC₅₀ value for acarbose in this study was 89.29 mg/mL, all tested samples demonstrated better inhibitory capacity than this control, supporting their potential as functional beverages.

Table 4. Inhibitory activity of the α -glucosidase of selected smoothie formulations before and after kombucha fermentation, and white tea and kombucha.

Sample	IC ₅₀ (mg/mL)
E5	80.61 ^c
E5F15	68.90 ^a
E10	84.46 ^d
<i>Camellia sinensis</i>	69.56 ^b
Kombucha	80.57 ^c

Note: E5: experiment 5, E10: experiment 10, E5F15: experiment 5 after 15 h of fermentation. ^{abcd}: Different letters in the same column indicate a significant difference between the samples at a 5% significance level according to Fisher's LSD test.

The inhibition of glucose and starch-hydrolyzing enzymes can be associated with the increase in total phenolic content observed after kombucha fermentation (up to 75.3%) [45]. Flavonoid glycosides may be responsible for inhibiting α -glucosidases [46]. Phenolic compounds are known to regulate carbohydrate metabolism primarily through the inhibition of α -glucosidase, which occurs via their capacity to chelate metal ions, alter enzyme conformation, and reduce catalytic efficiency. Additionally, polyphenols derived from apples have been shown to inhibit the intestinal sodium-glucose co-transporter 1 (SGLT1), thereby contributing to reduced glucose absorption and lower circulating glucose levels [47]. The combination of apples and berries may further enhance these effects, as the interaction among their phenolic compounds can provide synergistic health benefits. When incorporated into beverage systems, such interactions offer a promising strategy for the dietary management of hyperglycemia and its associated metabolic disorders [48].

4. Conclusions

The blend design allowed the development of ten beverages, among which two were most preferred by the sensory panel and selected for fermentation. Fermentation modified the characteristics of one formulation (E10) while improving the sensory attributes of the

other (E5), leading to the choice of only one fermented beverage (E5 fermented by 15 h) for subsequent physicochemical characterization, alongside its unfermented counterpart.

Although the unfermented smoothies exhibited higher overall antioxidant activity compared with the fermented sample, fermentation with kombucha microorganisms significantly influenced the chemical profile of the beverage. In the E5F15 formulation, fermentation reduced soluble solids, glucose, fructose, and caffeine content, while increasing titratable acidity, ascorbic acid, total phenolics, and specific compounds such as epicatechin, phloridzin, and chlorogenic acid. Additionally, the fermented beverage presented a more intense color and higher α -glucosidase inhibitory activity than the unfermented version.

This study highlights the development of smoothies, with or without fermentation by kombucha microorganisms, as a feasible approach that yielded products with distinctive sensory, nutritional, and functional characteristics, demonstrating strong potential for application in the functional beverage market. Nevertheless, further research is needed to assess the shelf-life and possible changes in bioactive compounds during storage.

Author Contributions: Formal analysis, L.S.Y. and P.R.L.; writing—original draft preparation, L.S.Y., A.A., A.N. and C.G.S.; writing—review and editing, A.N., C.G.S., T.E.F. and L.G.L.; methodology, L.S.Y., A.A.F.Z. and A.A.; supervision, A.N.; data curation and statistics, A.A.F.Z. and P.R.L.; resources, A.N.; project management, A.N.; fundraising, A.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Council for Scientific and Technological Development (CNPq) [grant N^o. 302797/2023-8; project N^o. 406799/2023-7], the Araucária Foundation (FA), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) [grant finance code 001].

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the State University of Ponta Grossa, Paraná, Brazil (CAAE no. 56282916.0.0000.0105; approval no. 1.649.931; Approval date 13 May 2016).

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

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors express their gratitude to the funding institutions and acknowledge the analytical infrastructure provided by the Apple Working Group (GTM) and the Multi-User Laboratory Complex (C-Labmu) at the State University of Ponta Grossa.

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

References

1. Škegro, M.; Putnik, P.; Bursać Kovačević, D.; Kovač, A.P.; Salkić, L.; Čanak, I.; Frece, J.; Zavadlav, S.; Ježek, D. Chemometric Comparison of High-Pressure Processing and Thermal Pasteurization: The Nutritive, Sensory, and Microbial Quality of Smoothies. *Foods* **2021**, *10*, 1167. [[CrossRef](#)]
2. Yassin, L.S.; Zielinski, A.A.F.; Alberti, A.; Los, P.R.; Demiate, I.M.; Simões, D.R.S.; Nogueira, A. An Overview of Brazilian Smoothies: From Consumer Profile to Evaluation of Their Physicochemical Composition, Bioactive Compounds, Antioxidant Activity and Sensory Description. *J. Food Bioact.* **2020**, *10*, 9–19. [[CrossRef](#)]
3. McCartney, D.M.; Rattray, M.; Desbrow, B.; Khalesi, S.; Irwin, C. Smoothies: Exploring the attitudes, beliefs and behaviours of consumers and non-consumers. *Curr. Res. Nutr. Food Sci. J.* **2018**, *6*, 425–436. [[CrossRef](#)]
4. Kovačević, D.B.; Brdar, D.; Fabečić, P.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. Strategies to achieve a healthy and balanced diet: Fruits and vegetables as a natural source of bioactive compounds. In *Agri-Food Industry Strategies for Healthy Diets and Sustainability*; Barba, F.J., Putnik, P., Kovačević, D.B., Eds.; Academic Press: London, UK, 2020; pp. 51–88.
5. Tlais, A.Z.A.; Trossolo, E.; Tonini, S.; Filannino, P.; Gobetti, M.; Di Cagno, R. Fermented Whey Ewe's Milk-Based Fruit Smoothies: Bio-Recycling and Enrichment of Phenolic Compounds and Improvement of Protein Digestibility and Antioxidant Activity. *Antioxidants* **2023**, *12*, 1091. [[CrossRef](#)]

6. Mali, S. *Kombucha Market Report 2025 (Global Edition)*, 8th ed.; Cognitive Market Research: Chicago, IL, USA, 2025; 250p.
7. Coton, M.; Pawtowski, A.; Taminiau, B.; Burgaud, G.; Deniel, F.; Coulloume-Labarthe, L.; Fall, A.; Daube, G.; Coton, E. Unraveling Microbial Ecology of Industrial-Scale Kombucha Fermentations by Metabarcoding and Culture-Based Methods. *FEMS Microbiol. Ecol.* **2017**, *93*, fix048. [[CrossRef](#)] [[PubMed](#)]
8. Wang, B.; Rutherford-Markwick, K.; Zhang, X.X.; Mutukumira, A.N. Kombucha: Production and Microbiological Research. *Foods* **2022**, *11*, 3456. [[CrossRef](#)]
9. Villarreal-Soto, S.A.; Beaufort, S.; Bouajila, J.; Souchard, J.P.; Taillandier, P. Understanding Kombucha Tea Fermentation: A Review. *J. Food Sci.* **2018**, *83*, 580–588. [[CrossRef](#)]
10. Jayabalan, R.; Malbasa, R.V.; Sathishkumar, M. Kombucha tea: Metabolites. In *Fungal Metabolites: Reference Series in Phytochemistry*; Merillon, J.M., Ramawat, K.G., Eds.; Springer International Publishing: Basel, Switzerland, 2021; pp. 1–14.
11. Vargas, B.K.; Fabricio, M.F.; Ayub, M.A.Z. Health Effects and Probiotic and Prebiotic Potential of Kombucha: A Bibliometric and Systematic Review. *Food Biosci.* **2021**, *44*, 101332. [[CrossRef](#)]
12. Miranda, J.F.; Ruiz, L.F.; Silva, C.B.; Uekane, T.M.; Silva, K.A.; Gonzalez, A.G.M.; Fernandes, F.F.; Lima, A.R. Kombucha: A Review of Substrates, Regulations, Composition, and Biological Properties. *J. Food Sci.* **2022**, *87*, 503–527. [[CrossRef](#)] [[PubMed](#)]
13. Carraro, M.M.; Sola, I.M.M.S.; Santos, R.D.M.; Demiate, I.M.; Alberti, A.; Nogueira, A. Impact of Heat Treatment on Hard Cider Enriched with Cryo-Concentrated Apple Must: Microbiological Profile, Functional Properties, and Storage Stability. *Fermentation* **2025**, *11*, 188. [[CrossRef](#)]
14. Zielinski, A.A.F.; Granato, D.; Alberti, A.; Nogueira, A.; Demiate, I.M.; Haminiuk, C.W.I. Modelling the Extraction of Phenolic Compounds and in Vitro Antioxidant Activity of Mixtures of Green, White and Black Teas (*Camellia sinensis* L. Kuntze). *J. Food Sci. Technol.* **2015**, *52*, 6966–6977. [[CrossRef](#)]
15. Bruns, R.E.; Scarminio, I.S.; Barros Neto, B. *Statistical Design—Chemometrics*; Elsevier: Amsterdam, The Netherlands, 2006.
16. Dairou, V.; Sieffermann, J.M. A Comparison of 14 Jams Characterized by Conventional Profile and a Quick Original Method, the Flash Profile. *J. Food Sci.* **2002**, *67*, 826–834. [[CrossRef](#)]
17. AOAC International. *Official Methods of Analysis of AOAC International*, 20th ed.; Latimer, G.W., Ed.; AOAC International: Rockville, MD, USA, 2016.
18. Santos, T.P.M.; Alberti, A.; Judacewski, P.; Zielinski, A.A.F.; Nogueira, A. Effect of Sulphur Dioxide Concentration Added at Different Processing Stages on Volatile Composition of Ciders. *J. Inst. Brew.* **2018**, *124*, 261–268. [[CrossRef](#)]
19. IAL (Instituto Adolfo Lutz). *Métodos Físico-Químicos Para Análise de Alimentos*; Instituto Adolfo Lutz: São Paulo, Brazil, 2008.
20. Alberti, A.; Zielinski, A.A.F.; Zardo, D.M.; Demiate, I.M.; Nogueira, A.; Mafra, L.I. Optimisation of the Extraction of Phenolic Compounds from Apples Using Response Surface Methodology. *Food Chem.* **2014**, *149*, 151–158. [[CrossRef](#)]
21. Rodriguez-Amaya, D.B. *A Guide to Carotenoid Analysis in Foods*; ILSI Press: Washington, DC, USA, 2001.
22. Zielinski, A.A.F.; Ávila, S.; Ito, V.; Nogueira, A.; Wosiacki, G.; Haminiuk, C.W.I. The Association between Chromaticity, Phenolics, Carotenoids, and In Vitro Antioxidant Activity of Frozen Fruit Pulp in Brazil: An Application of Chemometrics. *J. Food Sci.* **2014**, *79*, C510–C516. [[CrossRef](#)] [[PubMed](#)]
23. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [[CrossRef](#)]
24. Isabelle, M.; Lee, B.L.; Lim, M.T.; Koh, W.P.; Huang, D.; Ong, C.N. Antioxidant Activity and Profiles of Common Vegetables in Singapore. *Food Chem.* **2010**, *120*, 993–1003. [[CrossRef](#)]
25. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30. [[CrossRef](#)]
26. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [[CrossRef](#)]
27. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. [[CrossRef](#)]
28. Apak, R.; Güçlü, K.; Özyürek, M.; Çelik, S.E. Mechanism of Antioxidant Capacity Assays and the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) Assay. *Microchim. Acta* **2008**, *160*, 413–419. [[CrossRef](#)]
29. Silva, N.; Junqueira, V.C.A.; Silveira, N.F.A.; Taniwaki, M.H.; Gomes, R.A.R.; Okazaki, M.M. *Manual de Métodos de Análise Microbiológica de Alimentos e Água*; Editora Blucher: São Paulo, Brazil, 2018; ISBN 9788521212256.
30. Mccue, P.; Kwon, Y.I.; Shetty, K. Anti-amylase, anti-glucosidase and anti-angiotensin i-converting enzyme potential of selected foods. *J. Food Biochem.* **2005**, *29*, 278–294. [[CrossRef](#)]
31. Tuorila, H.M.; Meiselman, H.L.; Cardello, A.V.; Leshner, L.L. Effect of Expectations and the Definition of Product Category on the Acceptance of Unfamiliar Foods. *Food Qual. Prefer.* **1998**, *9*, 421–430. [[CrossRef](#)]
32. Anantachoke, N.; Duangrat, R.; Sutthiphatkul, T.; Ochaikul, D.; Mangmool, S. Kombucha Beverages Produced from Fruits, Vegetables, and Plants: A Review on Their Pharmacological Activities and Health Benefits. *Foods* **2023**, *12*, 1818. [[CrossRef](#)] [[PubMed](#)]

33. Sievers, M.; Lanini, C.; Weber, A.; Schuler-Schmid, U.; Teuber, M. Microbiology and Fermentation Balance in a Kombucha Beverage Obtained from a Tea Fungus Fermentation. *Syst. Appl. Microbiol.* **1995**, *18*, 590–594. [\[CrossRef\]](#)
34. Kallel, L.; Desseaux, V.; Hamdi, M.; Stocker, P.; Ajandouz, E.H. Insights into the Fermentation Biochemistry of Kombucha Teas and Potential Impacts of Kombucha Drinking on Starch Digestion. *Food Res. Int.* **2012**, *49*, 226–232. [\[CrossRef\]](#)
35. Managa, M.G.; Akinola, S.A.; Remize, F.; Garcia, C.; Sivakumar, D. Physicochemical Parameters and Bioaccessibility of Lactic Acid Bacteria Fermented Chayote Leaf (*Sechium edule*) and Pineapple (*Ananas comosus*) Smoothies. *Front. Nutr.* **2021**, *8*, 649189. [\[CrossRef\]](#)
36. Ojo, A.O.; Smidt, O. Microbial Composition, Bioactive Compounds, Potential Benefits and Risks Associated with Kombucha: A Concise Review. *Fermentation* **2023**, *9*, 472. [\[CrossRef\]](#)
37. Camacho, S.; Dop, M.; Graaf, C.; Stieger, M. Just Noticeable Differences and Weber Fraction of Oral Thickness Perception of Model Beverages. *J. Food Sci.* **2015**, *80*, S1583–S1588. [\[CrossRef\]](#)
38. Pratama, A.W.W.; Satyantini, W.H.; Kenconoajati, H. Crude Protein and Crude Fiber Content of Fish Silage Fermented with Kombucha Tea. *IOP Conf. Ser. Earth Environ. Sci.* **2023**, *1273*, 012079. [\[CrossRef\]](#)
39. McGuire, R.G. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255. [\[CrossRef\]](#)
40. Yang, W.; Liu, S.; Marsol-Vall, A.; Tähti, R.; Laaksonen, O.; Karhu, S.; Yang, B.; Ma, X. Chemical Composition, Sensory Profile and Antioxidant Capacity of Low-Alcohol Strawberry Beverages Fermented with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. *LWT* **2021**, *149*, 111910. [\[CrossRef\]](#)
41. Saquet, L.D.; Chim, J.F. Compostos Bioativos Da Amora-Preta (*Rubus* Spp.): Uma Revisão. *REBRAPA* **2020**, *11*, 35–53. [\[CrossRef\]](#)
42. Alves, R.O.; Monteiro, A.L.B.; Silva, C.M.; Silva, T.M.S.; Oliveira, R.L.; Porto, C.S.; Porto, T.S. Investigation of the Influence of Different *Camellia Sinensis* Teas on Kombucha Fermentation and Development of Flavored Kombucha with Brazilian Fruits. *Beverages* **2025**, *11*, 13. [\[CrossRef\]](#)
43. Shi, J.; Yang, G.; You, Q.; Sun, S.; Chen, R.; Lin, Z.; Simal-Gandara, J.; Lv, H. Updates on the Chemistry, Processing Characteristics, and Utilization of Tea Flavonoids in Last Two Decades (2001–2021). *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 4757–4784. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Kapp, J.M.; Sumner, W. Kombucha: A Systematic Review of the Empirical Evidence of Human Health Benefit. *Ann. Epidemiol.* **2019**, *30*, 66–70. [\[CrossRef\]](#)
45. Yangilar, F.; Gerek, M.D. Characterization of Rosemary (*Salvia rosmarinus*) Essential Oil Obtained by Solvent-Free Microwave Extraction with Kombucha Tea (*Anthriscus sylvestris* L.) Produced by Adding Guava (*Psidium guajava* L.) Peel and Pulp. *J. Agric. Sci.* **2025**, *31*, 33–45. [\[CrossRef\]](#)
46. Puspitasari, Y.E.; Tuenter, E.; Breynaert, A.; Foubert, K.; Herawati, H.; Hariati, A.M.; Aulanni'am, A.; De Bruyne, T.; Hermans, N. α -Glucosidase Inhibitory Activity of Tea and Kombucha from *Rhizophora mucronate* Leaves. *Beverages* **2024**, *10*, 22. [\[CrossRef\]](#)
47. Köpsel, M.; Kostka, T.; Rodriguez-Werner, M.; Esatbeyoglu, T. The Influence of Fruit Juice Extracts on Glucose Intestinal Transporters and Antioxidant Genes in a Caco-2 and HT29-MTX Co-Culture Cell System. *Food Funct.* **2025**, *16*, 1423–1441. [\[CrossRef\]](#)
48. Koutsos, A.; Riccadonna, S.; Ulaszewska, M.M.; Franceschi, P.; Trošt, K.; Galvin, A.; Braune, T.; Fava, F.; Perenzoni, D.; Mattivi, F.; et al. Two Apples a Day Lower Serum Cholesterol and Improve Cardiometabolic Biomarkers in Mildly Hypercholesterolemic Adults: A Randomized, Controlled, Crossover Trial. *Am. J. Clin. Nutr.* **2020**, *111*, 307–318. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.