fermentation

MDPI

Article

Development of Fermented Kombucha Tea Beverage Enriched with Inulin and B Vitamins

Yuliya Frolova [1,*], Valentina Vorobyeva [1], Irina Vorobyeva [1], Varuzhan Sarkisyan [1], Alexey Malinkin [2], Vasily Isakov [3] and Alla Kochetkova [1]

[1] Laboratory of Food Biotechnology and Foods for Special Dietary Uses, Federal State Budgetary Scientific Institution Federal Research Center of Nutrition, Biotechnology and Food Safety, 109240 Moscow, Russia
[2] Laboratory of Food Chemistry, Federal State Budgetary Scientific Institution Federal Research Center of Nutrition, Biotechnology and Food Safety, 109240 Moscow, Russia
[3] Department of Gastroenterology & Hepatology, Federal State Budgetary Scientific Institution Federal Research Center of Nutrition, Biotechnology and Food Safety, 109240 Moscow, Russia
* Correspondence: y.operarius@yandex.ru

Abstract: Kombucha is a sweet and sour beverage made by fermenting a liquid base with a symbiotic culture of bacteria and yeast. Different tea substrates, carbohydrate sources, and additional ingredients are used to create beverages with different physical and chemical characteristics. The purpose of this work was to create a recipe and technology to study the properties of the beverage based on kombucha with a given chemical composition. The content of added functional ingredients (vitamins and inulin) in quantities comparable with reference daily intake was the specified parameter characterizing the distinctive features of the enriched beverages. For fermentation using symbiotic cultures of bacteria and yeast, a black tea infusion sweetened with sucrose was used as a substrate. The changes in the physicochemical characteristics of the fermented tea beverage base were evaluated. The dynamics of changes in pH, acidity, the content of mono- and disaccharides, ethanol, organic acids, polyphenolic compounds, and volatile organic substances were shown. The fermentation conditions were selected (pH up to 3.3 ± 0.3, at T = 25 ± 1 °C, process duration of 14 days) to obtain the beverage base. Strawberry and lime leaves were used as flavor and aroma ingredients, and vitamins with inulin were used as functional ingredients. Since the use of additional ingredients changed the finished beverage's organoleptic profile and increased its content of organic acids, the final product's physical–chemical properties, antioxidant activity, and organoleptic indicators were assessed. The content of B vitamins in the beverages ranges from 29 to 44% of RDI, and 100% of RDI for inulin, which allows it to be attributed to the category of enriched products. The DPPH inhibitory activity of the beverages was 82.0 ± 7%, and the ethanol content did not exceed 0.43%. The beverages contained a variety of organic acids: lactic (43.80 ± 4.82 mg/100 mL), acetic (205.00 ± 16.40 mg/100 mL), tartaric (2.00 ± 0.14 mg/100 mL), citric (65.10 ± 5.86 mg/100 mL), and malic (45.50 ± 6.37 mg/100 mL). The technology was developed using pilot equipment to produce fermented kombucha tea enriched with inulin and B vitamins.

Keywords: fermented beverages; kombucha; black tea; physical and chemical characteristics; vitamins; inulin; volatile organic substances

Citation: Frolova, Y.; Vorobyeva, V.; Vorobyeva, I.; Sarkisyan, V.; Malinkin, A.; Isakov, V.; Kochetkova, A. Development of Fermented Kombucha Tea Beverage Enriched with Inulin and B Vitamins. *Fermentation* **2023**, *9*, 552. https://doi.org/10.3390/fermentation9060552

Academic Editor: Mutamed Ayyash

Received: 9 May 2023
Revised: 30 May 2023
Accepted: 6 June 2023
Published: 8 June 2023

1. Introduction

Kombucha is a beverage obtained through the fermentation of sweetened black or green tea using a symbiotic culture of bacteria and yeast (SCOBY) [1–3]. It is believed that the first references to the use of such a beverage date back to 220 BC in Manchuria [1,2,4]. The beneficial health properties attributed to kombucha have led to its present distribution in Europe, the United States, and other countries [4–6]. Kombucha is a popular beverage in many countries, as evidenced by the growth in its production, the constant expansion of its range, and numerous studies [7,8]. According to the results of a multidimensional scaling

of the results of articles searched using the "kombucha" keyword (Figure S1) obtained using the KH Coder software [9], several research areas can be identified: composition of symbiotic cultures of bacteria and yeast; factors influencing development and metabolism; the influence of technological parameters on quality and safety; antioxidant properties, including changes in polyphenolic composition during fermentation; and factors influencing the formation of bacterial cellulose, its application in various industries, and in vivo beverage studies.

Various studies of the kombucha production process and kombucha-based beverages are aimed at increasing the functional properties of the end product by selecting optimal production parameters (temperature [10,11], oxygen access [11], fermentation duration [10–12], the ratio of SCOBY to tea substrate, and size and shape of the fermentation tank [11]); basic ingredient composition (nitrogen sources [10,13–15], carbohydrate sources [13,16], and microbiological composition of SCOBY [17]); and additional flavor and functional ingredients [7,8,18].

An algorithm for the development of food products and the development of beverages includes the stage of the choice of ingredients based on the study of their chemical composition, properties, and physiological functions that provide health benefits. Apples [19,20], lemons [21], snake fruit [22], goji berry [15], cherry [23], dragon fruit [24], kiwi [24], red raspberry [25], blackthorn fruits [25], black carrot [25], strawberry tree [26], and others in fresh form, or as juices, purees, and extracts, are used as ingredients that form the taste and aroma of kombucha. [2,8]. Fresh and dried herbs and spices such as ginger [27], cherry leaf [28], sage [29,30], mint [29,31], linden [29], cinnamon [32], cardamom [32], thyme [30,32], echinacea [33], and functional ingredients [34] can be added at the stage of tea base preparation before its fermentation by SCOBY [31,32] or in the fermented base [7] for secondary fermentation and formation of the sensory profile of the beverage. These ingredients can be added at the stage of base preparation before fermentation by SCOBY [31,32] or in the fermented base [7] for secondary fermentation and formation of the sensory profile of the beverage. The use of combinations of ingredients allows not only to obtain a better taste of the beverages but also to regulate the composition of biologically active substances with beneficial health effects [35,36].

Along with the positive effects, kombucha also has negative effects associated with high acidity, in particular, the development of halitosis and potential negative effects on tooth enamel due to enamel degradation [37]. In this regard, it is reasonable to enrich kombucha in order to minimize its negative effects. Direct reduction of its acidity is not acceptable because a sour taste is expected and desired by the consumer. Therefore, searching for indirect ways to correct the negative effects of kombucha is an urgent task.

In order to provide additional health benefits, it is advisable to enrich kombucha-based beverages with physiologically important ingredients, which include soluble dietary fiber, prebiotics of polysaccharide nature, and vitamins.

Vitamins are essential micronutrients, which are low-molecular-weight organic compounds necessary for the enzymatic catalysis mechanisms, the normal course of metabolism, the maintenance of homeostasis, and the biochemical support of all vital functions of the body [38]. The need for vitamins is significantly influenced by a person's age, health condition, nature, and intensity of work. Inadequate consumption of vitamins is a risk factor for many nutrition-dependent diseases [39]. In this regard, it is advisable to include vitamins in the composition of beverages in sufficient amounts to correct their insufficient consumption.

Among the water-soluble ingredients are vitamin C and the complex of B vitamins, whose antioxidant effect neutralizes the reactive oxygen species (ROS) causative of many oral pathologies [40]. Vitamin C is the most common vitamin in kombucha, and its content in a single serving of kombucha varies within the reference daily intake (RDI). Supplementation with this vitamin is, therefore, not necessary. In turn, the content of B vitamins is an order of magnitude less than the RDI, so enrichment with vitamins of this group can increase the positive effects of kombucha consumption and is advisable [3].

In addition, for healthy bones and teeth, adequate dietary calcium intake is essential [41,42]. The direct introduction of soluble calcium salts will lead to an increase in pH

and consequently to a deterioration of the consumption properties of kombucha; therefore, the solution may be to use substances that increase the adsorption of calcium from the normal diet. This substance is inulin, a soluble dietary fiber (DF). The role of DF as a nutritional factor that actively influences metabolic processes in the human body has been repeatedly proven in a number of biological and clinical studies [43–45]. Among the proven physiological effects manifested by DF, the most pronounced include the normalization of the motor and evacuatory functions of the large intestine, prebiotic action, and influence on the state of lipid and carbohydrate metabolism. Soluble DF (alginates, pectin, β-glucans, gum arabic, some types of hemicelluloses, and modified cellulose) has a positive effect on lipid and carbohydrate metabolism [43,46], as does inulin. Inulin is contained in the tubers and roots of dahlias, artichokes, chicory, dandelion leaves, and asparagus; it is a mixture of oligomers and polymers of fructose and belongs to the group of fructosans [47,48]. As a prebiotic, inulin has a favorable effect on the human body in that it selectively stimulates the growth and activity of probiotic bacteria in the large intestine, thereby increasing the adaptive capabilities of the organism [47,49]. Besides stimulating the growth and activity of bifido- and lactobacilli, inulin increases calcium absorption in the large intestine, which has a positive effect on bone mineralization [47]. Inulin has a low glycemic index (from 4 to 7) and a low caloric value (1 kcal/g), as well as a distinctly sweet taste, neutral color, and odor [50]. This would replace some of the sucrose in the kombucha formulation, which would also have a positive effect on caries prevention with prolonged consumption of sugary beverages [41]. Inulin can also be used in the diet of patients with type 2 diabetes, as well as patients with impaired glucose tolerance. Similar to dietary fiber, inulin is resistant to the effects of digestive enzymes in the stomach and small intestine. This is due to their structure since, in the human body, there are no enzymes specific to the cleavage of bonds present in the molecules of such polysaccharides [50,51].

The introduction of these components can significantly change the properties of kombucha; therefore, the purpose of this work was to develop a kombucha containing vitamin B complex and inulin and evaluate its properties.

2. Materials and Methods

2.1. Materials

To obtain the fermented beverages, we used black Indian big leaf tea (Moscow, Russia), sugar (Moscow, Russia), a symbiotic culture of bacteria and yeast—SCOBY (Moscow, Russia), frozen strawberry fruit and Kaffir lime leaves (provided by a local supermarket), inulin (90% of the main substance) (Belgium), and vitamin premix RUS 28,174 by DSM Nutritional Products Europe Ltd. (Basel, Switzerland) (the composition is shown in Table 1). For analytical studies, we used NaOH, Folin-Ciocalteu reagent (Sigma-Aldrich), gallic acid (98%) (Diaem), Na_2CO_3, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (ABCR GmbH and Co. KG, Karlsruhe, Germany). Analytical grade reagents (Sigma-Aldrich, St. Louis, MO, USA) were used for chromatography.

Table 1. Composition of vitamin premix.

Compound	Concentration, mg/kg
Thiamine (Vitamin B_1)	48,750
Riboflavin	44,000
Pyridoxine (Vitamin B_6)	57,000
Niacin	479,998
Folic acid	7500

2.2. Fermented Beverage Base Production Technology

Black tea (0.5 wt.%) was added to boiled water at 94 ± 2 °C and brewed for 10 min with occasional stirring. Then, sugar was added in an amount of 5.0 wt.% and stirred for

5 min until complete dissolution. The obtained solution was filtered to remove tea leaves and cooled to 23 ± 2 °C. Then, the SCOBY culture was added (10.0 wt.%), and the container was covered with a permeable cloth. Fermentation was carried out in 25 L fermenters (Brew Bucket Brewmaster, Pittsburg, CA, USA). The duration of the fermentation process was 14 days at 25 ± 1 °C. After fermentation, the resulting base was filtered through a filter (70 µm) and pasteurized at 72 ± 2 °C for 40 min. The resulting base was then cooled to a temperature of 25–30 °C and used to prepare a beverage or stored in hermetically sealed containers at 5 °C until use. During fermentation with a periodicity of 0 days, 3 days, 7 days, and 14 days, acidity, pH, total content of polyphenolic compounds, content of mono- and disaccharides, organic acids, ethanol, and the profile of volatile substances were controlled.

2.3. Methods

2.3.1. Titratable Acidity Determination

The acidity value was determined using the titrimetric method [52]. The method is based on titration with NaOH solution (0.1 N) of all acidic substances. Before the study, the samples were freed from carbon dioxide formed during fermentation by boiling. Acidity was expressed in cubic centimeters of sodium hydroxide solution with a concentration of 1 N, which was used to titrate 100 mL of the beverages.

2.3.2. pH Determination

The pH changes were monitored using an electronic pH meter S20_K Mettler Toledo (Greifensee, Switzerland) [31].

2.3.3. Determination of Carbohydrates Profile

The composition of carbohydrates (mono- and disaccharides) was determined using standard high-performance liquid chromatography on an Agilent Technologies 1260 chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a refractometric detector Agilent 1260 RID, G1362A (Agilent Technologies, Santa Clara, CA, USA) according to [53]. Before analysis, the samples were centrifuged at 990× *g* for 15 min (if necessary, diluted with water at a ratio of 1:5 (by volume)). In order to determine the mass concentration of sucrose, glucose, and fructose, the samples were diluted with distilled water in a 1:20 ratio. Then, 1–2 mL of the sample was taken and filtered through a filter with a 0.45 µm pore diameter into a vial.

2.3.4. Determination of the Dry Matter Content

Total soluble substances were determined using an Atago refractometer (Tokyo, Japan) and expressed in degrees Brix (°Brix), according to [54].

2.3.5. Determination of Ethanol Content

The determination of ethanol content in the samples was carried out on an Agilent Technologies 7890A gas chromatograph with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA) and an analytical column Supelcowax 10 (60 m × 0.53 mm × 1 µm) from Supelco (Bellefonte, PA, USA), according to [55] with modifications. Temperature program: 78 °C for 2 min, heating to 110 °C at 3 °C/min, rising to 220 °C at 30 °C/min, with a 10 min delay. The carrier gas was helium, and the flow rate was 2.8 mL/min. A 20 mL vial was placed on the scale, 5 mL of water was added, 2 mL of sample was added (weight of the added sample was recorded with an accuracy of 1 mg), then 1 mL of internal standard solution (0.8% 1-butanol solution, the weight of internal standard solution was recorded with an accuracy of 1 mg) was added, and another 10 mL of water was added. After being stirred, 2 mL was taken into a 2 mL centrifuge tube and centrifuged at 18,400× *g*. Then, 1 mL was taken into a 2 mL glass vial, and 2 mL was injected into the chromatograph. The chromatograph was calibrated in the concentration range of 3 to 300 µg/g.

2.3.6. Determination of Organic Acids

Organic acids were determined by standard reversed-phase high-performance liquid chromatography on an Agilent Technologies 1100 chromatograph (Agilent Technologies, Santa Clara, CA, USA), according to [56]. Organic acids were separated in a chromatographic column filled with octadecyl silica gel. Concentrations were determined using a spectrophotometric detector at 210 nm using the external standard method.

2.3.7. Determination of Total Polyphenol Content

The total content of polyphenolic compounds during fermentation was determined by the Folin-Ciocalteu spectrophotometric method using a SpectroQuest 2800 spectrophotometer (UNICO, Suite E, Dayton, NJ, USA), according to [57] with modifications. Before analysis, the beverage sample was diluted with distilled water in a ratio of 1:9. To 1 mL of the sample, 5 mL of 10% Folin-Ciocalteu solution was added and incubated for 5 min. After this time, 4 mL of 7.5% Na_2CO_3 solution was added, thoroughly mixed, and incubated in a dark place for 1 h. The optical density of the samples was measured at 765 nm. Gallic acid was used as a standard.

2.3.8. Determination of Volatile Substances

The analysis was performed on an Agilent Technologies 7890A gas chromatograph with an Agilent Technologies 5975C mass detector (Agilent Technologies, Santa Clara, CA, USA) and a Supelcowax 10 60 m × 0.53 mm × 1 µm chromatographic column (Supelco, Bellefonte, PA, USA), according to [58] with modifications. Divinylbenzene/carboxene/polydimethylsiloxane (50/30 µm) fiber with manual holder (Supelco, Bellefonte, PA, USA) was used for the extraction of aroma components. The fiber was preconditioned before the analyses, according to the instructions of the manufacturer. A total of 10 mL of sample was placed in a 20 mL headspace vial, sealed with a septum and an aluminum cap. The fiber was placed in the space above the sample and incubated for 30 min on a tile heated to 110 °C. Then, it was placed into the gas chromatograph injector, and the analysis was performed. Temperature program: 35 °C for 5 min, heating to 220 °C at a rate of 4 °C/min, isotherm 40 min. Helium was used as a carrier gas. Injector was operated in splitless mode at a temperature of 225 °C. Operation parameters of the mass detector: scanning range 35–400 m/z, ionization source temperature 230 °C, quadrupole temperature 150 °C, and electron impact ionization with energy 70 eV. The results were processed using the program "MSD ChemStation E02.02.1431" (Agilent Technologies, Santa Clara, CA, USA), the program "The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library Ver. 2.0 gm" (National Institute of Standards and Technology, Gaithersburg, MD, USA), and a set of commercially available mass spectral libraries. Components with a spectral coefficient of agreement with the library of more than 700 were considered to be identified. Subsequent processing of the obtained data was performed using the "Microsoft Office Excel 2007 SP3 MSO" software package.

2.3.9. Modeling of Reaction Kinetics

An analysis of reaction kinetics was performed according to the principle outlined in [59]. In order to determine the reaction rate constant (k), the conformity of changes in the concentration of volatile substances with the models of zero (Equation (1)), first (Equation (2)), and second (Equation (3)) order reactions were evaluated:

$$C - C_0 = kt \quad (1)$$

$$\ln\left(\frac{C}{C_0}\right) = kt \quad (2)$$

$$\frac{1}{C} - \frac{1}{C_0} = kt \quad (3)$$

where C_0 is the initial concentration (%), C is the concentration (%) at time t (days), and k is the reaction constant.

The value of the reaction constant was calculated using the linear equation of the relationship between concentration and reaction time. In addition to the reaction constant, we also determined the half-life ($t_{1/2}$) of the studied substances according to Equations (4) and (5) for first- and second-order reactions, respectively.

$$t_{1/2} = \frac{\ln 2}{k} \quad (4)$$

$$t_{1/2} = \frac{1}{k} \cdot \frac{1}{C_0} \quad (5)$$

2.4. The Technology of Fermented Beverage Production

Before use, the prepared fermented base (technology of obtaining described above) was brought to a temperature of 25–30 °C. Preliminary organoleptic studies (data not shown) allowed us to identify preferences and determine the number of ingredients that form the taste and aroma of fermented beverages: frozen strawberries and lime leaves. Strawberries and lime leaves were crushed, added to the prepared fermented base, and left for 24 h at 23 ± 2 °C for infusion and secondary fermentation. Further filtration was carried out in stages from 70 μm to 5 μm. For enrichment, pre-dissolved inulin and vitamin premix, which included water-soluble B vitamins B_1, B_2, B_6, PP, and folic acid, were added to the beverages after filtration. Then, the beverages were placed into a cylindroconical tank "SS Brewtech Unitank" (USA) equipped with a circulating cooler "Termex" and a bottling nozzle (Tomsk, Russia) for carbonization of the beverages (10 °C, 1.1 MPa) with subsequent bottling into glass bottles. To stop further fermentation and ensure microbiological purity, the bottles with the beverages were pasteurized (72 ± 2 °C, 40 min) in a Binder BD53 (Tuttlingen, Germany). The line with the pilot equipment is shown in Figure 1.

Figure 1. Pilot equipment line for the production of fermented beverages.

The finished beverages were characterized according to the indicators: acidity, pH, total soluble substances, the content of mono-, disaccharides, ethanol, organic acids (the methods for determining the indicators are presented above), inulin, vitamins, antioxidant activity, and organoleptic evaluation.

2.5. Methods for Investigation of Fermented Beverages

2.5.1. Determination of Inulin Content

Inulin content in the finished beverages was determined with a standard method using high-performance liquid chromatography on an Agilent Technologies 1260 (Agilent Technologies, Santa Clara, CA, USA) chromatograph with refractometric detector Agilent 1260 RID, G1362A (Agilent Technologies) [60].

2.5.2. Determination of Vitamin Content

The content of vitamins B_1, B_2, B_6, PP, and folic acid was determined according to the method [61].

2.5.3. Assessment of Antioxidant Activity

The study of the antioxidant activity of the finished beverages was carried out using free radical DPPH [62]. Before the study, the beverages were filtered and diluted twice. The filtered beverages in an amount of 100 µL were mixed with 2 mL of DPPH solution in ethanol (250 µM) and incubated in a dark place for 1 h at 24 ± 1 °C. The obtained solutions were studied on an electron paramagnetic resonance spectrometer SPINSCAN X (Republic of Belarus) operating at a frequency of X-band. Conditions for obtaining electron paramagnetic resonance (EPR) spectra: central field 336 mT, sweep amplitude 15 mT, modulation amplitude 200 µT, and power 20 dB. The obtained spectra were processed using the program e-Spinoza (Republic of Belarus). The percent inhibition of the EPR spectrum was calculated according to the following equation: % inhibition = $[(I_0 - I)/I0] \times 100\%$, where I_0 is the area of the EPR spectrum of DPPH (control sample), and I is the area of the EPR spectrum of DPPH with sample.

2.5.4. Organoleptic Evaluation

The sensory assessors were selected, trained, and monitored according to ISO 8586 standards [63,64]. The sensory panel consisted of 8 trained assessors (5 females and 3 males; mean age: 37.2 ± 1.3). They were required to be healthy and not smoke, drink coffee, or eat spicy food for 3 h before analysis.

The conditions of the sensory evaluation environment, including the area of sample preparation, sensory evaluation, and concentrative discussion, met the requirements of the ISO 8589 standard [65]. The sensory evaluation area was kept in an adequately air-conditioned environment, and the temperature in the booths was controlled at about 25 °C. The conventional sensory profile of the kombucha samples was established according to the ISO 13299 standard [66]. Organoleptic evaluation of the finished beverages was carried out on a 5-point scale by indicators: appearance, smell, consistency, taste, and color.

2.6. Statistical Processing

Obtained data are presented in the form of average values. Statistical data processing and graphing were carried out using the software package R studio (version 3.5.3) [67], using the module ggplot2 [68]. The Kruskal–Wallis test with the Mann–Whitney test as post hoc was used to compare changes of measured parameters during the fermentation (the first day was used as control) [69]. Results are shown as H and *p*-values. All measurements were conducted in triplicate. p values < 0.05 were considered significant.

3. Results and Discussion

Studies on kombucha and beverages based on it are mainly conducted on a laboratory scale, with volumes ranging from 200 mL to 2 L [11]. However, a number of researchers studied the fermentation process in larger volumes [70,71], and it was shown that regardless of the size and volume of the vessel, with a constant value of the interfacial surface, it is possible to obtain a beverage with similar properties [11]. In order to optimize the industrial production of enriched kombucha, the main fermentation process was carried out in 25 L fermenters.

3.1. Preparation and Study of Fermented Beverage Bases

The processes of obtaining fermented tea beverages have their own technological features, which depend on cultural traditions, the raw materials used, the concentration of tea and sugar, and the duration of fermentation [72]. However, the basic technological steps are common and consist of brewing the tea, adding sucrose or other carbohydrate sources, filtering and cooling to room temperature, adding SCOBY and/or starter fluid (fermented kombucha from a previous batch), and fermentation [72,73]. The fermentation process of sweetened tea begins after the addition of SCOBY and/or starter liquids. Under the action of enzymes formed in yeast cells, hydrolysis of sucrose to glucose and fructose occurs at room temperature, which are subsequently converted to ethanol and carbon dioxide as a result of alcoholic fermentation [74]. The change in the profile of carbohydrates during fermentation is shown in Figure 2.

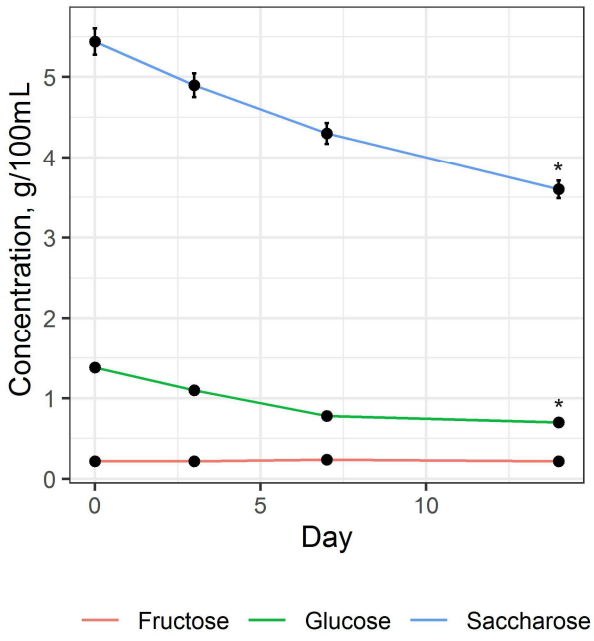

Figure 2. Changes in carbohydrate profile during fermentation (significant ($p < 0.05$) differences from the starting point are shown by "*" symbol).

Figure 2 shows that during the fermentation process for 14 days, there is a decrease in saccharose content by an average of 45% (H = 8.897, $p = 0.045$). The concentration of glucose also significantly decreased (H = 9.051, $p = 0.029$), but the change in the fructose concentration had only an insignificant tendency (H = 0.723, $p = 0.868$), indicating the different metabolism of these carbohydrates by SCOBY microorganisms. The change in the concentration of glucose and fructose during fermentation depends on the composition of the SCOBY [5]. Thus, acetic acid bacteria assimilate fructose and glucose to produce various organic acids and form a cellulose film [74], while the assimilation of glucose and fructose is not the same [75]. Yeast mainly assimilates glucose with the formation of carbon dioxide and ethanol; however, yeast of the genus *Saccharomyces* prefers glucose, whereas certain yeast of the genus *Zygosaccharomyces* prefer fructose. In our study, the fermentation process was continued for 14 days, after which the fermented base contained residual sugars. However, there are alternative methods. For example, prolonged fermentation for more than 30 days allows the use of a fermented base with 0% sugar content, due to which its caloric value is lower than that of a base containing sugar. For the preparation of the beverages, the base is necessarily diluted with juices, herbal infusions, etc., while for sweetening, stevia extract or other sweeteners can be used. In our study, the criterion for the completion of the primary fermentation process was the pH value. Residual sugar in the studied base gave a sour-sweet taste and reduced the perception of the sour taste of the

beverages. During fermentation, due to the accumulation of organic acids, mainly acetic acid, the pH value decreases, and the acidity increases [74]. Control of these indicators allows for the preparation of a beverage with the desired organoleptic characteristics. The results of changes in pH and acidity during the fermentation of the beverage base are shown in Figure 3.

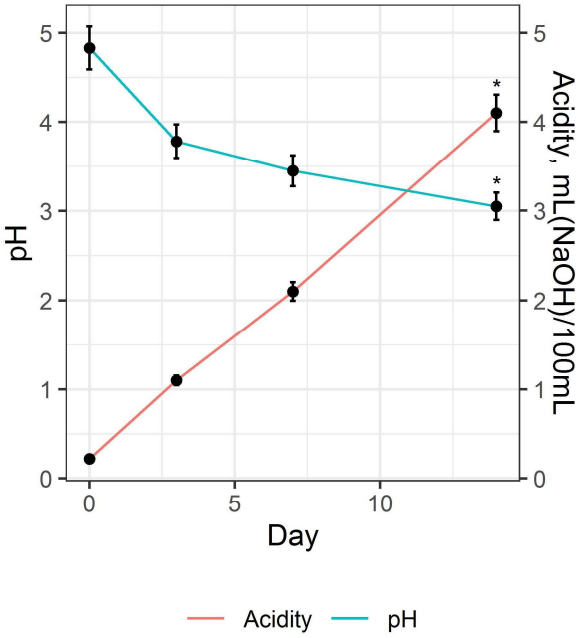

Figure 3. Changes in pH and acidity of the beverage base during fermentation (significant ($p < 0.05$) differences from the starting point are shown by "*" symbol).

As shown in Figure 3, the rate of change in the pH and acidity of the beverage base during fermentation is non-linear. At the beginning of the fermentation process (up to 3 days), there is an intense decrease in pH values with a subsequent decrease in rate ($H = 13.5$, $p = 0.0091$). At the same time, the change in acidity is reversed: during the first 7 days, acidity increases slightly, then increases sharply, which is associated with the accumulation of organic acids in the substrate, in particular acetic acid (Figure 4). It should be noted that the sharp increase in acidity ($H = 13.5$, $p = 0.0091$) does not have a strong correlation ($r = -0.864$, $p < 0.001$) correlate with the change in pH value, which may be due to the manifestation of buffering properties of the fermented beverages [76]. Fermentation was continued until reaching pH values of 3.3 ± 0.3.

Due to alcoholic fermentation, ethanol was formed during the first stages of the fermentation process [74]. It was found that the ethanol content on day 7 of fermentation was 0.12%, and on day 14, it did not exceed 0.22%. When selecting a tea substrate for kombucha, the dynamics of ethanol accumulation during fermentation should be taken into account [77,78].

The fermentation process begins with the hydrolysis of sucrose into fructose and glucose by enzymes produced in the yeast cells. Part of the glucose and fructose is then used by the yeast to produce ethanol and carbon dioxide [11,79]. Another part of the fructose and glucose and the resulting ethanol is used by acetic acid bacteria to produce cellulose and organic acids, of which acetic acid is the main one [11,79]. In addition to acetic acid, other organic acids, such as gluconic acid, lactic acid, malic acid, citric acid, tartaric acid, and others, are accumulated in much smaller amounts during fermentation [80]. Regulation of the concentration and type of organic acids formed is achieved by varying the component composition of the tea base and carbohydrate sources [81,82]. In this work, to obtain a fermented beverage base, traditional raw materials were used: black tea and

sugar; therefore, the main organic acid formed in the process of fermentation was acetic acid (Figure 4).

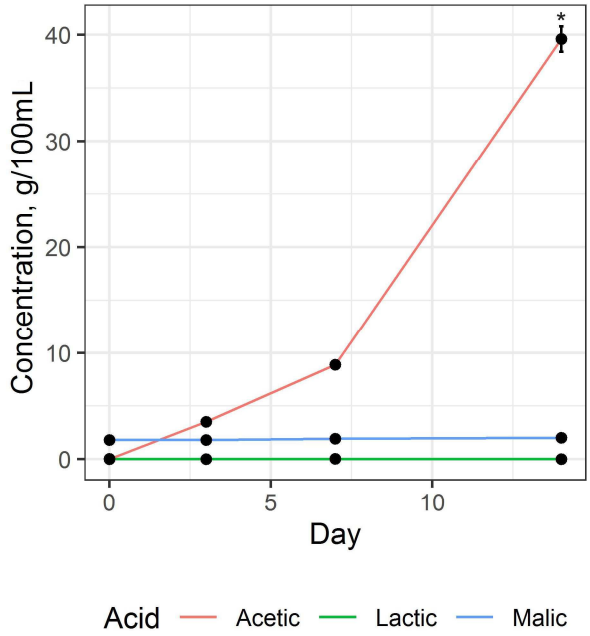

Figure 4. Changes in the content of the main organic acids during fermentation (significant ($p < 0.05$) differences from the starting point are shown by "*" symbol).

It was found that the accumulation of acetic acid in the first three days of fermentation occurs slowly, and subsequently, the rate of accumulation increases (H = 10.531, $p = 0.015$). The character of the curve of acetic acid accumulation correlates with the change in the acidity of the base during fermentation (Figure 3). In the period from the 10th to the 14th day, there is an accumulation of acetic acid, which negatively affects the organoleptic properties of the base due to the appearance of a sharp smell and sour taste. Accumulation of other major organic acids was much lower and was statistically significant for lactic acid (H = 9.581, $p = 0.023$) and insignificant for malic acid (H = 1.049, $p = 0.789$).

Consumption of beverages with a high organic acid content is unsafe and poses potential risks to consumers. To eliminate the negative influence of the temperature factor on the technological process and the formation of undesirable fermentation products in fermenters, they were maintained at a temperature of 25 ± 1 °C.

The popularity of fermented tea beverages is due not only to their original taste and odor but also to their health-promoting properties, in particular, their antioxidant properties. The biological activity of SCOBY is primarily related to the chemical composition of the tea itself used in the preparation of the beverage [6]. Flavonoids such as flavanols (flavan-3-ols), flavonols, flavones, flavanones, and anthocyanidins are the main components of tea leaves, which together account for up to 30% of the dry weight of tea leaves [83]. The composition of polyphenolic compounds and their content depend on the type of tea, growing conditions, processing and storage technology, etc. [84]. The results of the evaluation of the total content of polyphenolic compounds in the beverage base and their change during fermentation are shown in Figure 5.

During the fermentation process under the influence of SCOBY during the first 7 days, there is an increase in the total content of polyphenolic compounds (H = 7.308, $p = 0.042$). As the process continues, their content decreases. Data from other researchers support our conclusions [85]. The transformation of phenolic compounds from their conjugated forms into their free forms, as well as the oxidation and dimerization of tea catechins with the increased catalytic ability of polyphenol oxidase, are all linked to changes in this indicator during fermentation [85]. The conversion of flavonoids during fermentation, e.g.,

thearubigin to theaflavin, leads to a change in the color of the fermented base from dark to light [86].

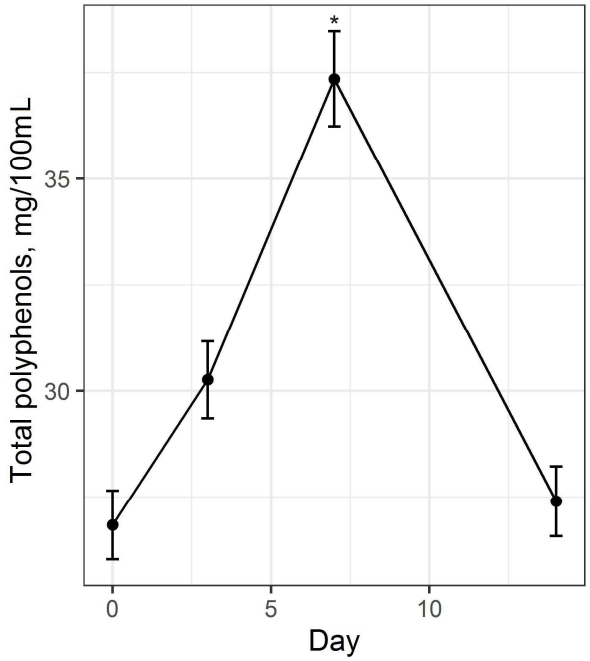

Figure 5. Changes in the content of total polyphenolic compounds during fermentation in terms of gallic acid (significant ($p < 0.05$) differences from the starting point are shown by "*" symbol).

The type of tea plant, growing conditions, and a variety of other factors affect how the flavor profile of the finished product develops [87]. In the process of fermentation, there is the formation of volatile compounds that form a specific category of beverages' flavor profile. As a result of this research, 70 volatile compounds were identified in the initial infusion of black tea before the beginning of the fermentation process. On the 14th day of fermentation (the completion of the fermentation process), 55 volatile compounds were identified. From the total list of identified volatile organic compounds, substances with a high content in the aromatic profile were selected. These substances were subdivided into four main groups: aldehydes, acids, alcohols, and terpenes (Figure 6).

During the fermentation, the profile of volatile substances changes: some identified in the initial tea infusion disappeared while the concentration of others increased. As can be seen from Figure 6, at the starting point, aldehydes and terpenes have the highest concentrations in the tea base, with hexanal (12.1%) and linalool (8.2%) predominating in these groups, respectively. The presence of hexanal and linalool imparts an herbaceous and floral aroma to the beverages, which disappears during fermentation. On the third day of fermentation, due to the alcoholic fermentation, the content of ethanol in the vapor phase increases rapidly (59%). On the 7th day, the content of organic acids increases significantly, mainly due to acetic (13.5%), pentanoic (4.3%), and octanoic (3.1%) acids. On the 7th day, the concentration of alcohols (phenylethyl, ethanol, dimethylphenol, and 3-methyl-1-butanol) decreases naturally. The maximum content was observed for ethanol (40%). On the 14th day of the study, the alcohol content decreased to below 3%, except for ethanol, where the concentration in the vapor phase above the fermented beverage sample was 35.2%. However, the ethanol content in the fermented base was 0.22%. A low alcohol content of up to 1% may not be felt by the consumer because the threshold of taste for ethanol ranges from 1 to 2% [88], while a concentration of more than 1% may result in a bitter taste. The content of acetic acid on the 14th day increased to 23.4%, and the content of 3-methylbutanoic acid also increased significantly. The results obtained are generally characteristic of the process of tea fermentation by SCOBY [89] and indicate that aldehydes and terpenes are the main volatile organic compounds that are metabolized in

the fermentation process. This may be an indication of the loss of the traditional black tea aroma in the final product.

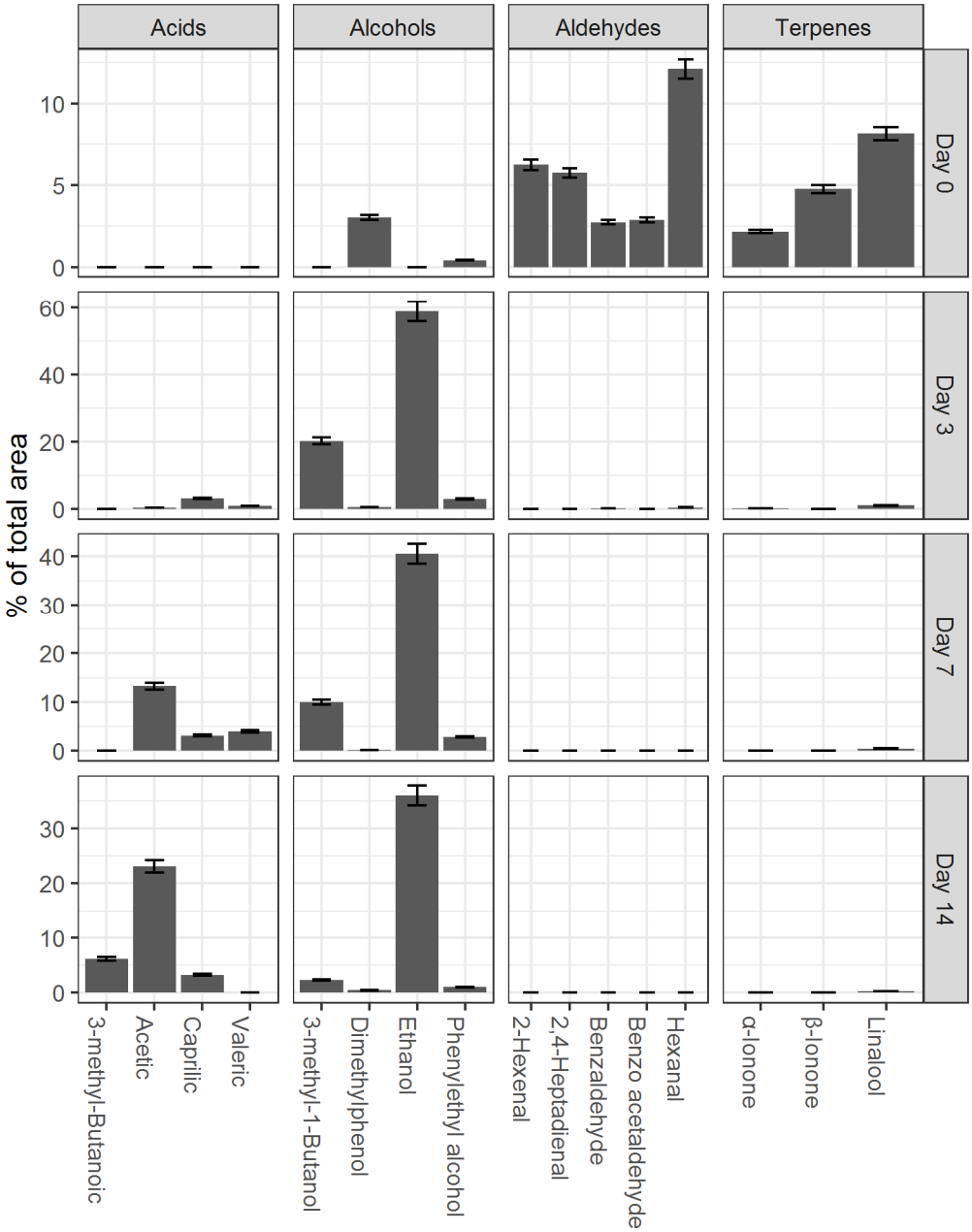

Figure 6. Diagram of the content of groups of volatile organic substances in the studied samples from the first to the fourteenth day of fermentation.

The kinetics of the accumulation and degradation of volatile substances in kombucha were analyzed. For substances whose content was measured throughout the experiment and varied at selected measurement intervals, information on the order and constants of reactions as well as on the time of semi-transformation of substances was obtained (Table 2). The table shows information on the models with the highest values of linear correlation coefficients (r^2).

Only the accumulation of acetic acid fitted well ($r^2 = 0.960$) with the zero-order reaction kinetic model. This indicates that the process of vinegar synthesis from ethanol under the action of acetic acid bacteria does not depend on ethanol concentration. Thus, we can say that up to the 14th day of fermentation, ethanol was in excess for the synthesis of acetic

acid. This may be due, in particular, to the low rate of metabolization of ethanol, which has a half-life time of 18.05 days. The decrease in ethanol (after the third day) proceeds as a second-order reaction with the lowest reaction constant among those studied. In contrast to ethanol, the change in the content of 3-methyl-1-butanol proceeds faster as a first-order reaction with a half-life of 1.15 days.

Table 2. Kinetic parameters of degradation or synthesis of volatile compounds in kombucha.

Compound	Reaction Order	r^2	K	$t_{1/2}$, Days
Acetic acid	Zero	0.960 ± 0.012	1.22 ± 0.03 ($M \cdot d^{-1}$)	-
Ethanol	Second	0.854 ± 0.010	$9.39 \pm 0.11 \times 10^{-4}$ ($M^{-1} \cdot d^{-1}$)	18.05 ± 1.28
3-methyl-1-Butanol	First	0.999 ± 0.014	$5.99 \pm 0.02 \times 10^{-1}$ (d^{-1})	1.15 ± 0.16
Linalool	Second	0.996 ± 0.013	$2.95 \pm 0.07 \times 10^{-1}$ ($M^{-1} \cdot d^{-1}$)	0.42 ± 0.09
Hexanal	Second	0.984 ± 0.021	1.4 ± 0.06 ($M^{-1} \cdot d^{-1}$)	0.06 ± 0.01

"-"—not applicable.

The decrease in linalool and hexanal, which were initially present in the tea, also corresponds to the kinetics of the second-order reaction. They are characterized by a high rate of degradation: 0.42 days for linalool and 0.06 days for hexanal. The rate constants of these reactions are 3–4 orders of magnitude higher than the rate constant of the ethanol metabolization reaction. Nevertheless, the order of these reactions indicates that the decrease in the concentration of linalool and hexanal is not a simple process but is caused by a number of factors, such as the action of aldehyde dehydrogenase in acetic acid bacteria [90].

3.2. Preparation and Study of the Fermented Beverage

A kombucha base was used as a matrix for the beverages. The use of fresh berries, fruits, and vegetables in the composition of beverages is due to their content of vitamins, minerals, polyphenolic compounds, phenolic acids, tannins, etc. [91,92]. Lime leaves give a special flavor to the product due to the essential oils. Crushed strawberries and lime leaves were introduced into the prepared fermented base and incubated at 23 ± 2 °C for 24 h for infusion and secondary fermentation, followed by filtration. The composition of the fermented strawberry and lime beverages is shown in Table 3.

Table 3. Composition of the fermented strawberry and lime beverages.

Ingredient	Amount, g
Kombucha base	1038.0
Strawberry	173.0
Kaffir lime leaf	3.7
Total	1214.7
Yield after filtration	1000

The addition of flavoring ingredients leads to secondary fermentation and the extraction of biologically active substances. The effect of the addition of flavoring ingredients (strawberry fruit and lime leaves) and secondary fermentation on the physicochemical parameters of the fermented base is presented in Table 4.

Based on the data obtained (Table 4), it was found that the addition of ingredients leads to secondary fermentation, as evidenced by the decrease in sucrose content and increase in alcohol and organic acids. Changing the profile of organic acids is associated not only with the process of secondary fermentation but also with the extraction of acids from strawberry fruits. According to [93,94], strawberry fruits mainly contain citric and malic

acids and, in small amounts, tartaric, oxalic, and fumaric acids. The increase in the content of citric, malic, and tartaric acids in the fermented beverages after the second fermentation relative to the fermented base (Table 4) indicates their extraction from strawberry fruits. The increase in alcohol content (from 0.22 to 0.43%) and decrease in sugar content (from 3.6 g/100 mL to 0.18 g/100 mL) indicate the progress of alcoholic fermentation. There is also a dramatic increase in acetic acid content after secondary fermentation. At the same time, the pH value increased insignificantly. As noted earlier, the change in pH is not always associated with a change in the content of organic acids because the fermented base may have buffering properties [76].

Table 4. Comparison of physicochemical parameters of fermented base and base after secondary fermentation.

Parameter	Value	Value
	Kombucha Base	**Fermented Beverages**
Acidity, mL 1 mole/L NaOH/100 mL	4.10 ± 0.26	4.72 ± 0.38
pH	3.31 ± 0.08	3.57 ± 0.07
Solids content, °Brix	4.60 ± 0.25	4.95 ± 0.25
Ethanol content, %	0.22 ± 0.01	0.43 ± 0.01
Content of organic acids, mg/100 mL		
- lactic	0.025 ± 0.001	43.80 ± 4.82
- acetic	39.80 ± 1.82	205.00 ± 16.40
- tartaric	-	2.00 ± 0.14
- citric	-	65.10 ± 5.86
- malic	2.04 ± 0.11	45.50 ± 6.37
Carbohydrates contents (mono-, disaccharides), g/100 mL		
- glucose	0.70 ± 0.11	1.45 ± 0.13
- fructose	0.22 ± 0.12	1.47 ± 0.12
- saccharose	3.60 ± 0.04	0.18 ± 0.02

"-"—not detected.

In order to increase the nutritional value of the beverages, they were enriched with B vitamins and inulin, nutrients with clinically proven health benefits. Inulin was pre-dissolved in boiled water at 60–70 °C, and then the solution was cooled to room temperature. We added vitamin premix and stirred until it completely dissolved. When calculating the number of enriching ingredients, we proceeded from the recommendation to ensure the content in a beverage portion (220 g) of inulin was in the amount corresponding to the RDI for vitamins, from 15 to 50% of the RDI. The composition of the enriched fermented beverages is shown in Table 5.

Table 5. Composition of the enriched fermented beverage.

Ingredient	Amount, g
Filtered fermented beverage	903.34
Water	84.0
Inulin	12.6
Vitamin premix	0.06
Total	1000

The content of B vitamins in the enriched fermented beverages was as follows: vitamin B_1—0.27 ± 0.02 mg/100 g, vitamin B_2—0.25 ± 0.02 mg/100 g, vitamin B_6—0.34 ± 0.03 mg/100 g, folic acid—0.04 ± 0.01 mg/100 g, and vitamin PP—2.76 ± 0.22 mg/100 g. Inulin content was 1.21 ± 0.12%. In the manufacturing process of enriched food products, vitamins are subjected to physical and chemical treatment, which may adversely affect their safety. The results of analytical studies have stated that the technological processes of beverage production, in particular carbonization and pasteurization, have a negative impact on the stability of folic acid (12% loss).

The antioxidant properties of tea and tea beverages are influenced by their high polyphenolic compound content [6]. The DPPH inhibitory activity of the kombucha base was 89.9%. According to the proposed technology (Section 2.4), crushed strawberry fruits and lime leaves were added to the base, which can lead to the extraction of substances that have antioxidant properties. At the same time, according to the recipe (Table 3), dissolved inulin was added to the kombucha base, which could lead to dilution of the base and a slight decrease in antioxidant activity. Moreover, the technology of obtaining enriched fermented beverages involves a pasteurization stage (72 ± 2 °C, 40 min), in which the destruction of biologically active substances that have antioxidant properties may occur. Analysis of the enriched fermented beverages showed that the DPPH inhibitory activity was 82.0%.

Consumer properties of beverages are decisive for successful market promotion (or sale). In the finished product, the ethanol content did not exceed 0.43%, which classifies the developed beverages as non-alcoholic [95]. Organoleptic properties of the developed beverages were formed as a result of fermentation processes as well as the extraction of flavor and aromatic substances from introduced strawberries and lime leaves. Characteristics of the organoleptic and the characteristics of the developed enriched beverages are presented in Table 6.

Table 6. Organoleptic characteristics of kombucha base and fermented beverages.

Parameter	Description	Value	
		Kombucha Base	Fermented Beverages
Appearance	Non-transparent liquid. Sludge due to the characteristics of the raw material used is allowed, without foreign inclusions.	5.00 ± 0.00	5.00 ± 0.00
Color	Red with a brownish hue due to the color of the raw material used.	5.00 ± 0.00	5.00 ± 0.00
Odor	Inherent in the ingredients used with the aroma of a fermented beverage.	4.25 ± 0.71	4.71 ± 0.48
Taste	Sweet and sour with strawberry flavor and pronounced kaffir lime leaf flavor.	4.25 ± 0.71	4.71 ± 0.48
Consistency	Liquid with low viscosity and rich body.	4.38 ± 0.50	5.00 ± 0.00

The results of the study of organoleptic properties, shown in Table 6, show a high estimate of the developed beverages by the indicators of “odor”, “consistency”, and “taste”.

At the stage of secondary fermentation, there is an extraction from the introduced ingredients of flavors, aromatics, sugars, vitamins, minerals, polyphenols, and other biologically active substances. This technological stage is very important for the formation of the organoleptic properties of the beverages. The resulting enriched fermented beverages have a pleasant color and exquisite aroma, not characteristic of traditional beverages. However, the dominant flavor and aroma of lime were noted by some tasters as a drawback.

The content of vitamins in a portion (220 g) of a developed beverage refers to enriched products. Inulin content does not exceed the upper allowable level of consumption (Figure 7).

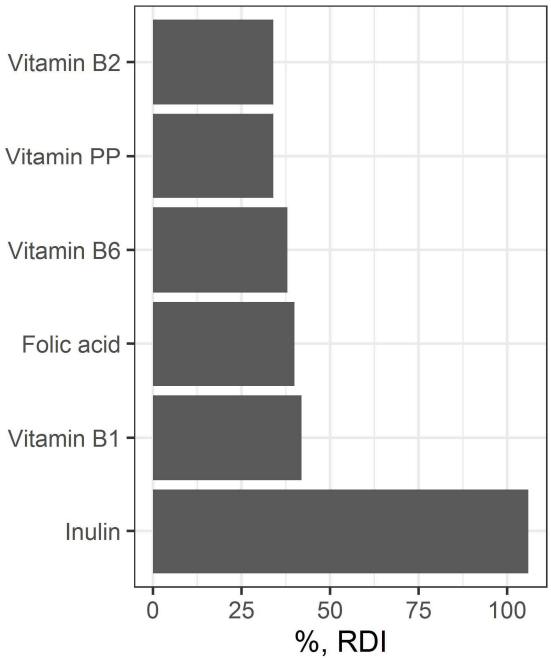

Figure 7. Meeting the average daily requirement of an adult's inulin and vitamins.

The technological scheme for producing kombucha enriched with inulin and B vitamins was developed (Figure 8).

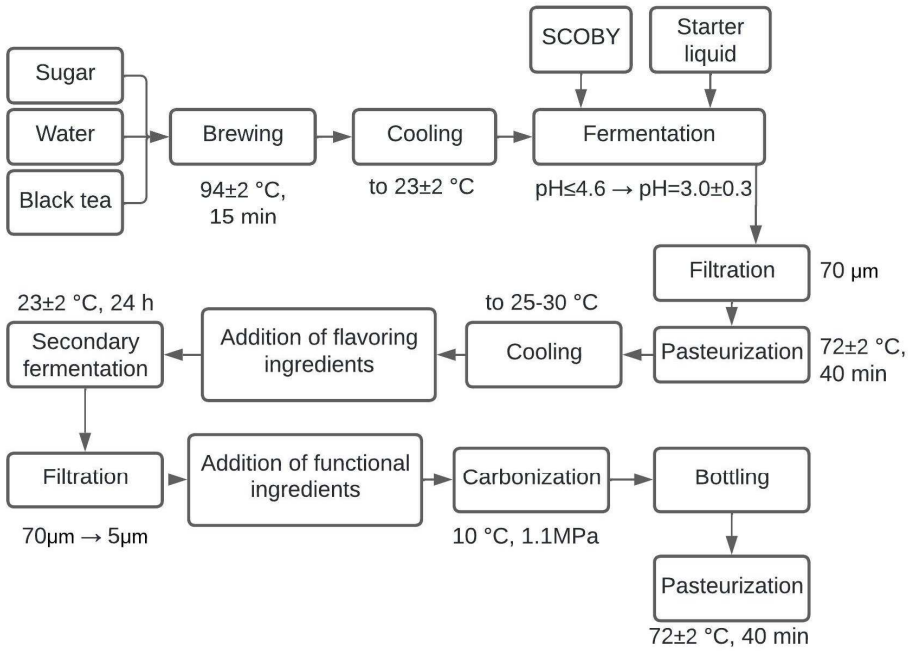

Figure 8. Technological scheme of enriched fermented beverage production.

Basic and auxiliary processes in fermented beverage technology can be distinguished [73]. The developed technology of obtaining fermented beverage kombucha includes the main stages: brewing tea, cooling, fermentation, and filtration, i.e., stages that allow obtaining classic unpasteurized fermented beverage without the additional introduction of other ingredients. The auxiliary stages of the process (pasteurization, addition of flavors and functional ingredients, secondary fermentation, and carbonation) allow for a broader range of beverages and additional health benefits. A clinical study of the developed beverage enriched with inulin and vitamins presented in [96] showed a decrease in the intensity of the complaints

significant for irritable bowel syndrome with constipation due to the normalization of stool frequency and consistency.

4. Conclusions

Changes in physical and chemical indicators of the tea base sweetened with sucrose under the influence of a symbiotic culture of bacteria and yeast have been investigated. The conditions for obtaining fermented tea bases with stable physicochemical characteristics on the pilot equipment were described. The change in the profile of volatile flavor-forming substances in the beverages during fermentation has been studied, and the kinetics of their accumulation have been described. The formulation and technology of fermented tea beverage kombucha with strawberry and lime enriched with vitamins and inulin were developed. As practical recommendations for the preservation of vitamins during long storage, it is recommended to bottle the developed fermented beverage kombucha, enriched with vitamins and inulin, into dark glass bottles. The results of these studies testify that kombucha can serve as a basis for new specialized beverages with tailored consumer properties, and the addition of B vitamins and inulin leads to an acceptable organoleptic profile.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9060552/s1, Figure S1: Results of multidimensional scaling of the search results for articles on the keyword "kombucha".

Author Contributions: Conceptualization, A.K. and V.I.; methodology, Y.F., V.V., I.V. and V.S.; validation, V.V., I.V. and V.S.; formal analysis, Y.F. and A.M.; writing—original draft preparation, Y.F., V.S., V.V. and I.V.; writing—review and editing, A.K., V.S. and Y.F.; visualization, V.S. and Y.F.; supervision, A.K.; project administration, V.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, grant number 19-76-30014.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Nyhan, L.M.; Lynch, K.M.; Sahin, A.W.; Arendt, E.K. Advances in kombucha tea fermentation: A review. *Appl. Microbiol.* **2022**, *2*, 73–103. [CrossRef]
2. Bortolomedi, B.M.; Paglarin, C.S.; Brod, F.C.A. Bioactive compounds in kombucha: A review of substrate effect and fermentation conditions. *Food Chem.* **2022**, *385*, 132719. [CrossRef] [PubMed]
3. de 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]
4. Soares, M.G.; de Lima, M.; Schmidt, V.C.R. Technological aspects of kombucha, its applications and the symbiotic culture (SCOBY), and extraction of compounds of interest: A literature review. *Trends Food Sci. Technol.* **2021**, *110*, 539–550. [CrossRef]
5. Antolak, H.; Piechota, D.; Kucharska, A. Kombucha tea—A double power of bioactive compounds from tea and symbiotic culture of bacteria and yeasts (SCOBY). *Antioxidants* **2021**, *10*, 1541. [CrossRef] [PubMed]
6. 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]
7. Kim, J.; Adhikari, K. Current trends in kombucha: Marketing perspectives and the need for improved sensory research. *Beverages* **2020**, *6*, 15. [CrossRef]
8. Frolova, Y.V. Russian market of fermented kombucha beverages. *Vopr. Pitan.* **2022**, *91*, 115–118. [CrossRef]
9. Higuchi, K. A Two-Step Approach to Quantitative Content Analysis: KH Coder Tutorial using Anne of Green Gables (Part II). *Ritsumeikan Soc. Sci. Rev.* **2017**, *53*, 137–147.
10. Li, S.; Zhang, Y.; Gao, J.; Li, T.; Li, H.; Mastroyannis, A.; He, S.; Rahaman, A.; Chang, K. Effect of Fermentation Time on Physiochemical Properties of Kombucha Produced from Different Teas and Fruits: Comparative Study. *J. Food Qual.* **2022**, *2022*, 2342954. [CrossRef]

11. 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] [PubMed]
12. La Torre, C.; Fazio, A.; Caputo, P.; Plastina, P.; Caroleo, M.C.; Cannataro, R.; Cione, E. Effects of long-term storage on radical scavenging properties and phenolic content of Kombucha from black tea. *Molecules* **2021**, *26*, 5474. [CrossRef] [PubMed]
13. Kluz, M.I.; Pietrzyk, K.; Pastuszczak, M.; Kacaniova, M.; Kita, A.; Kapusta, I.; Zaguła, G.; Zagrobelna, E.; Struś, K.; Marciniak-Lukasiak, K.; et al. Microbiological and physicochemical composition of various types of homemade kombucha beverages using alternative kinds of sugars. *Foods* **2022**, *11*, 1523. [CrossRef] [PubMed]
14. Vitas, J.S.; Cvetanović, A.D.; Mašković, P.Z.; Švarc-Gajić, J.V.; Malbaša, R.V. Chemical composition and biological activity of novel types of kombucha beverages with yarrow. *J. Funct. Foods* **2018**, *44*, 95–102. [CrossRef]
15. Abuduaibifu, A.; Tamer, C.E. Evaluation of physicochemical and bioaccessibility properties of goji berry kombucha. *J. Food Process. Preserv.* **2019**, *43*, e14077. [CrossRef]
16. Francisco, Á.R.; Igor, H. Development of a no added sugar kombucha beverage based on germinated corn. *Int. J. Gastron. Food Sci.* **2021**, *24*, 100355. [CrossRef]
17. Li, R.; Xu, Y.; Chen, J.; Wang, F.; Zou, C.; Yin, J. Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha. *LWT* **2022**, *155*, 112937. [CrossRef]
18. da Silva Júnior, J.C.; Magnani, M.; da Costa, W.K.A.; Madruga, M.S.; Olegário, L.S.; Borges, G.D.S.C.; Dantas, A.M.; Lima, M.S.; de Lima, L.C.; de Limo Brinto, I.; et al. Traditional and flavored kombuchas with pitanga and umbu-caja pulps: Chemical properties, antioxidants, and bioactive compounds. *Food Biosci.* **2021**, *44*, 101380. [CrossRef]
19. Zubaidah, E.; Yurista, S.; Rahmadani, N.R. Characteristic of physical, chemical, and microbiological kombucha from various varieties of apples. *IOP Conf. Ser. Earth Environ. Sci.* **2018**, *131*, 012040. [CrossRef]
20. Liamkaew, R.; Chattrawanit, J.; Danvirutai, P. Kombucha production by combinations of black tea and apple juice. *Prog. Appl. Sci. Technol.* **2016**, *6*, 139–146.
21. Zazueta, A.C.L.; Zavala, P.G.; Arguilez, C.G.Z. una Estandarización química de una bebida fermentada de Kombucha a base de té verde, té de limón ye infusión hojas de guayaba. *Rev. De Investig. Académica Sin Front. Div. Cienc. Económicas Soc.* **2022**, *38*. [CrossRef]
22. Zubaidah, E.; Dewantari, F.J.; Novitasari, F.R.; Srianta, I.; Blanc, P.J. Potential of snake fruit (*Salacca zalacca* (Gaerth.) Voss) for the development of a beverage through fermentation with the Kombucha consortium. *Biocatal. Agric. Biotechnol.* **2018**, *13*, 198–203. [CrossRef]
23. Yavari, N.; Assadi, M.M.; Larijani, K.; Moghadam, M.B. Response surface methodology for optimization of glucuronic acid production using kombucha layer on sour cherry juice. *Aust. J. Basic Appl. Sci.* **2010**, *4*, 3250–3256.
24. Tang, Z.; Zhao, Z.; Chen, S.; Lin, W.; Wang, Q.; Shen, N.; Qin, Y.; Xiao, Y.; Chen, H.; Bu, T.; et al. Dragon fruit-kiwi fermented beverage: In vitro digestion, untargeted metabolome analysis and anti-aging activity in *Caenorhabditis elegans*. *Front. Nutr.* **2022**, *9*, 1052818. [CrossRef] [PubMed]
25. Ulusoy, A.; Tamer, C.E. Determination of suitability of black carrot (*Daucus carota* L. spp. *sativus* var. *atrorubens* Alef.) juice concentrate, cherry laurel (*Prunus laurocerasus*), blackthorn (*Prunus spinosa*) and red raspberry (*Rubus ideaus*) for kombucha beverage production. *J. Food Meas. Charact.* **2019**, *13*, 1524–1536.
26. Tejedor-Calvo, E.; Morales, D. Chemical and Aromatic Changes during Fermentation of Kombucha Beverages Produced Using Strawberry Tree (*Arbutus unedo*) Fruits. *Fermentation* **2023**, *9*, 326. [CrossRef]
27. Budiari, S.; Mulyani, H.; Maryati, Y.; Filailla, E.; Devi, A.F.; Melanie, H. Chemical properties and antioxidant activity of sweetened red ginger extract fermented with kombucha culture. *Agrointek: J. Teknol. Ind. Pertan.* **2023**, *17*, 60–69. [CrossRef]
28. Nintiasari, J.; Ramadhani, M.A. Uji Kuantitatif flavonoid dan Aktivitas Antioksidan Teh Kombucha Daun Kersen (*Muntingia calabura*). *Indones. J. Pharm. Nat. Prod.* **2022**, *5*, 174–183.
29. Kayisoglu, S.; Coskun, F. Determination of physical and chemical properties of kombucha teas prepared with different herbal teas. *Food Sci. Technol.* **2020**, *41*, 393–397. [CrossRef]
30. Velićanski, A.; Cvetković, D.; Markov, S. Characteristics of Kombucha fermentation on medicinal herbs from Lamiaceae family. *Rom. Biotechnol. Lett.* **2013**, *18*, 8034–8042.
31. Tanticharakunsiri, W.; Mangmool, S.; Wongsariya, K.; Ochaikul, D. Characteristics and upregulation of antioxidant enzymes of kitchen mint and oolong tea kombucha beverages. *J. Food Biochem.* **2021**, *45*, e13574. [CrossRef] [PubMed]
32. Shahbazi, H.; Hashemi Gahruie, H.; Golmakani, M.T.; Eskandari, M.H.; Movahedi, M. Effect of medicinal plant type and concentration on physicochemical, antioxidant, antimicrobial, and sensorial properties of kombucha. *Food Sci. Nutr.* **2018**, *6*, 2568–2577. [CrossRef]
33. Özyurt, H. Changes in the content of total polyphenols and the antioxidant activity of different beverages obtained by Kombucha 'tea fungus'. *Int. J. Agric. Environ. Food Sci.* **2020**, *4*, 255–261. [CrossRef]
34. Tu, C.; Tang, S.; Azi, F.; Hu, W.; Dong, M. Use of kombucha consortium to transform soy whey into a novel functional beverage. *J. Funct. Foods* **2019**, *52*, 81–89. [CrossRef]
35. Martínez Leal, J.; Valenzuela Suárez, L.; Jayabalan, R.; Huerta Oros, J.; Escalante-Aburto, A. A review on health benefits of kombucha nutritional compounds and metabolites. *CyTA-J. Food* **2018**, *16*, 390–399. [CrossRef]
36. Kapp, J.M.; Sumner, W. Kombucha: A systematic review of the empirical evidence of human health benefit. *Ann. Epidemiol.* **2019**, *30*, 66–70. [CrossRef] [PubMed]

37. Bishop, P.; Pitts, E.R.; Budner, D.; Thompson-Witrick, K.A. Chemical composition of kombucha. *Beverages* **2022**, *8*, 45. [CrossRef]
38. Godswill, A.G.; Somtochukwu, I.V.; Ikechukwu, A.O.; Kate, E.C. Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. *Int. J. Food Sci.* **2020**, *3*, 1–32. [CrossRef]
39. Kodentsova, V.M.; Vrzhesinskaya, O.A.; Nikityuk, D.V.; Tutelyan, V.A. Vitamin status of adult population of the Russian Federation: 1987-2017. *Vopr. Pitan.* **2018**, *87*, 62–68.
40. Pietrangelo, L.; Magnifico, I.; Petronio Petronio, G.; Cutuli, M.A.; Venditti, N.; Nicolosi, D.; Perna, A.; Guerra, G.; Di Marco, R. A Potential "Vitaminic Strategy" against Caries and Halitosis. *Appl. Sci.* **2022**, *12*, 2457. [CrossRef]
41. Gondivkar, S.M.; Gadbail, A.R.; Gondivkar, R.S.; Sarode, S.C.; Sarode, G.S.; Patil, S.; Awan, K.H. Nutrition and oral health. *Disease-a-Month* **2019**, *65*, 147–154. [CrossRef] [PubMed]
42. Sewón, L.A.; Karjalainen, S.M.; Söderling, E.; Lapinleimu, H.; Simell, O. Associations between salivary calcium and oral health. *J. Clin. Periodontol.* **1998**, *25*, 915–919. [CrossRef] [PubMed]
43. Lattimer, J.M.; Haub, M.D. Effects of dietary fiber and its components on metabolic health. *Nutrients* **2010**, *2*, 1266–1289. [CrossRef] [PubMed]
44. Myhrstad, M.C.; Tunsjø, H.; Charnock, C.; Telle-Hansen, V.H. Dietary fiber, gut microbiota, and metabolic regulation—Current status in human randomized trials. *Nutrients* **2020**, *12*, 859. [CrossRef]
45. Murga-Garrido, S.M.; Hong, Q.; Cross, T.W.L.; Hutchison, E.R.; Han, J.; Thomas, S.P.; Vivas, E.I.; Denu, J.; Ceschin, D.G.; Tang, Z.Z.; et al. Gut microbiome variation modulates the effects of dietary fiber on host metabolism. *Microbiome* **2021**, *9*, 117. [CrossRef]
46. Pyryeva, E.A.; Safronova, A.I. The role of dietary fibers in the nutrition of the population. *Vopr. Pitan.* **2019**, *88*, 5–11.
47. Wan, X.; Guo, H.; Liang, Y.; Zhou, C.; Liu, Z.; Li, K.; Niu, F.; Zhai, X.; Wang, L. The physiological functions and pharmaceutical applications of inulin: A review. *Carbohydr. Polym.* **2020**, *246*, 116589. [CrossRef]
48. Redondo-Cuenca, A.; Herrera-Vázquez, S.E.; Condezo-Hoyos, L.; Gómez-Ordóñez, E.; Rupérez, P. Inulin extraction from common inulin-containing plant sources. *Ind. Crops Prod.* **2021**, *170*, 113726. [CrossRef]
49. Illippangama, A.U.; Jayasena, D.D.; Jo, C.; Mudannayake, D.C. Inulin as a functional ingredient and their applications in meat products. *Carbohydr. Polym.* **2022**, *275*, 118706. [CrossRef]
50. Gupta, N.; Jangid, A.K.; Pooja, D.; Kulhari, H. Inulin: A novel and stretchy polysaccharide tool for biomedical and nutritional applications. *Int. J. Biol. Macromol.* **2019**, *132*, 852–863. [CrossRef]
51. Ni, D.; Xu, W.; Zhu, Y.; Zhang, W.; Zhang, T.; Guang, C.; Mu, W. Inulin and its enzymatic production by inulosucrase: Characteristics, structural features, molecular modifications and applications. *Biotechnol. Adv.* **2019**, *37*, 306–318. [CrossRef] [PubMed]
52. Lončar, E.S.; Kanurić, K.G.; Malbaša, R.V.; Đurić, M.S.; Milanović, S.D. Kinetics of saccharose fermentation by Kombucha. *Chem. Ind. Chem. Eng. Q.* **2014**, *20*, 345–352. [CrossRef]
53. Bokov, D.O.; Sergunova, E.V.; Marakhova, A.I.; Morokhina, S.L.; Plakhotnaia, O.N.; Krasnyuk, I.I.; Bessonov, V.V. Determination of Sugar Profile in Viburnum Fruits and its Dosage Forms by HPLC-RID. *Pharmacogn. J.* **2020**, *12*, 103–108. [CrossRef]
54. Gamboa-Gómez, C.I.; Simental-Mendía, L.E.; González-Laredo, R.F.; Alcantar-Orozco, E.J.; Monserrat-Juarez, V.H.; Ramírez-España, J.C.; Gallegos-Infante, J.A.; Moreno-Jiménez, M.R.; Rocha-Guzmán, N.E. In vitro and in vivo assessment of anti-hyperglycemic and antioxidant effects of Oak leaves (*Quercus convallata* and *Quercus arizonica*) infusions and fermented beverages. *Food Res. Int.* **2017**, *102*, 690–699. [CrossRef] [PubMed]
55. Borai, A.; Sebaih, S.; Alshargi, A.; Albarzan, F.; Al-Ghamdi, A.; Al-Ghamdi, S.; Boraie, S.; Bahijri, S.; Al-Shareef, A.S.; Al-Armi, A.; et al. Ethanol content of a traditional Saudi beverage Sobia. *Int. J. Food Prop.* **2021**, *24*, 1790–1798. [CrossRef]
56. Tytelyan, V.A. *Guidance on Methods of Quality Control and Safety of Biologically Active Food Supplements*; Federal Center for State Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia: Moscow, Russia, 2004; 240p.
57. Chu, S.C.; Chen, C. Effects of origins and fermentation time on the antioxidant activities of kombucha. *Food Chem.* **2006**, *98*, 502–507. [CrossRef]
58. Zou, C.; Li, R.Y.; Chen, J.X.; Wang, F.; Gao, Y.; Fu, Y.Q.; Xu, Y.Q.; Yin, J.F. Zijuan tea-based kombucha: Physicochemical, sensorial, and antioxidant profile. *Food Chem.* **2021**, *363*, 130322. [CrossRef]
59. Espenson, J.H. *Chemical Kinetics and Reaction Mechanisms*; McGraw-Hill: New York, NY, USA, 1995; p. 296.
60. Bokov, D.O.; Khromchenkova, E.P.; Sokurenko, M.S.; Vasilev, A.V.; Bessonov, V.V. Development of a technique for the determination of inulin in natural instant chicory after enzymatic hydrolysis by high-performance liquid chromatography. *Vopr. Pitan.* **2017**, *86*, 50–55.
61. Bendryshev, A.A.; Pashkova, E.B.; Pirogov, A.V.; Shpigun, O.A. Determination of water-soluble vitamins in vitamin premixes, bioacitve dietary supplements, and pharmaceutical preparations using high-efficiency liquid chromatography with gradient elution. *Mosc. Univ. Chem. Bull.* **2010**, *65*, 260–268. [CrossRef]
62. Polak, J.; Bartoszek, M.; Chorążewski, M. Antioxidant capacity: Experimental determination by EPR spectroscopy and mathematical modeling. *J. Agric. Food Chem.* **2015**, *63*, 6319–6324. [CrossRef]
63. *ISO 8586-1:1996*; Sensory Analysis-General Guidance for the Selection, Training and Monitoring of Assessors-Part 1: Selected Assessors. ISO: Geneva, Switzerland, 1996.
64. *ISO 8586-2:1996*; Sensory Analysis-General Guidance for the Selection, Training and Monitoring of Assessors-Part 2: Expert Sensory Assessors. ISO: Geneva, Switzerland, 1996.

65. *ISO 8589:2007*; General Guidance for the Design of Test Rooms. International Organization for Standardization. ISO: Geneva, Switzerland, 2007.
66. *ISO 13299:2016*; Sensory Analysis. Methodology. General Guidance for Establishing a Sensory Profile. International Organization for Standardization (ISO): Geneva, Switzerland, 2016.
67. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020; Available online: https://www.R-project.org/ (accessed on 5 June 2023).
68. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016.
69. Granato, D.; de Araújo Calado, V.M.; Jarvis, B. Observations on the use of statistical methods in food science and technology. *Food Res. Int.* **2014**, *55*, 137–149. [CrossRef]
70. Cvetković, D.; Markov, S.; Djurić, M.; Savić, D.; Velićanski, A. Specific interfacial area as a key variable in scaling-up Kombucha fermentation. *J. Food Eng.* **2008**, *85*, 387–392. [CrossRef]
71. Malbaša, R.; Lončar, E.; Djurić, M.; Klašnja, M.; Kolarov, L.J.; Markov, S. Scale-up of black tea batch fermentation by kombucha. *Food Bioprod. Process.* **2006**, *84*, 193–199. [CrossRef]
72. Diez-Ozaeta, I.; Astiazaran, O.J. Recent advances in Kombucha tea: Microbial consortium, chemical parameters, health implications and biocellulose production. *Int. J. Food Microbiol.* **2022**, *377*, 109783. [CrossRef]
73. Vorobyeva, V.M.; Vorobyeva, I.S.; Sarkisyan, V.A.; Frolova, Y.V.; Kochetkova, A.A. Technological features of fermented beverages production using kombucha. *Vopr. Pitan.* **2022**, *91*, 115–120. [CrossRef]
74. Tran, T.; Grandvalet, C.; Verdier, F.; Martin, A.; Alexandre, H.; Tourdot-Maréchal, R. Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 2050–2070. [CrossRef]
75. Chen, C.; Liu, B.Y. Changes in major components of tea fungus metabolites during prolonged fermentation. *J. Appl. Microbiol.* **2000**, *89*, 834–839. [CrossRef]
76. Jayabalan, R.; Subathradevi, P.; Marimuthu, S.; Sathishkumar, M.; Swaminathan, K. Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chem.* **2008**, *109*, 227–234. [CrossRef]
77. Jakubczyk, K.; Kałduńska, J.; Kochman, J.; Janda, K. Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea. *Antioxidants* **2020**, *9*, 447. [CrossRef]
78. Gaggìa, F.; Baffoni, L.; Galiano, M.; Nielsen, D.S.; Jakobsen, R.R.; Castro-Mejía, J.L.; Bosi, S.; Truzzi, F.; Musumeci, F.; Dinelli, G.; et al. Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients* **2018**, *11*, 1. [CrossRef]
79. Laureys, D.; Britton, S.J.; De Clippeleer, J. Kombucha tea fermentation: A review. *J. Am. Soc. Brew. Chem.* **2020**, *78*, 165–174. [CrossRef]
80. Jayabalan, R.; Malbaša, R.V.; Sathishkumar, M. Kombucha tea: Metabolites. In *Fungal Metabolites*; Springer: Cham, Switzerland, 2017; pp. 965–978.
81. Reiss, J. Influence of different sugars on the metabolism of the tea fungus. *Z. Für Lebensm.-Unters. Und-Forsch.* **1994**, *198*, 258–261. [CrossRef]
82. Kaewkod, T.; Bovonsombut, S.; Tragoolpua, Y. Efficacy of kombucha obtained from green, oolong, and black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on colorectal cancer cell line. *Microorganisms* **2019**, *7*, 700. [CrossRef]
83. Liu, Z.; Bruins, M.E.; de Bruijn, W.J.; Vincken, J.P. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. *J. Food Compos. Anal.* **2020**, *86*, 103385. [CrossRef]
84. Meng, X.H.; Li, N.; Zhu, H.T.; Wang, D.; Yang, C.R.; Zhang, Y.J. Plant resources, chemical constituents, and bioactivities of tea plants from the genus *Camellia* section *Thea*. *J. Agric. Food Chem.* **2018**, *67*, 5318–5349. [CrossRef] [PubMed]
85. Wang, S.; Zhang, L.; Qi, L.; Liang, H.; Lin, X.; Li, S.; Yu, C.; Ji, C. Effect of synthetic microbial community on nutraceutical and sensory qualities of kombucha. *Int. J. Food Sci. Technol.* **2020**, *55*, 3327–3333. [CrossRef]
86. Chakravorty, S.; Bhattacharya, S.; Chatzinotas, A.; Chakraborty, W.; Bhattacharya, D.; Gachhui, R. Kombucha tea fermentation: Microbial and biochemical dynamics. *Int. J. Food Microbiol.* **2016**, *220*, 63–72. [CrossRef] [PubMed]
87. Feng, Z.; Li, Y.; Li, M.; Wang, Y.; Zhang, L.; Wan, X.; Yang, X. Tea aroma formation from six model manufacturing processes. *Food Chem.* **2019**, *285*, 347–354. [CrossRef] [PubMed]
88. Bishop, P.; Pitts, E.R.; Budner, D.; Thompson-Witrick, K.A. Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chem. Adv.* **2022**, *1*, 100025. [CrossRef]
89. Jayabalan, R.; Marimuthu, S.; Swaminathan, K. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem.* **2007**, *102*, 392–398. [CrossRef]
90. Matsushita, K.; Adachi, O. Quinoprotein aldehyde dehydrogenases in microorganisms. In *Principles and Applications of Quinoproteins*; CRC Press: Boca Raton, FL, USA, 2020; pp. 65–72.
91. Akimov, M.Y.; Bessonov, V.V.; Kodentsova, V.M.; Eller, K.I.; Vrzhesinskaya, O.A.; Beketova, N.A.; Kosheleva, O.V.; Bogachuk, M.N.; Malinkin, A.D.; Makarenko, M.A.; et al. Biological value of fruits and berries of Russian production. *Vopr. Pitan.* **2020**, *89*, 220–232. [PubMed]
92. Tutel'ian, V.A.; Lashneva, N.V. Biologically active substances of plant origin. Flavonols and flavones: Prevalence, dietary sourses and consumption. *Vopr. Pitan.* **2013**, *82*, 4–22. [PubMed]

93. Ikegaya, A.; Toyoizumi, T.; Ohba, S.; Nakajima, T.; Kawata, T.; Ito, S.; Arai, E. Effects of distribution of sugars and organic acids on the taste of strawberries. *Food Sci. Nutr.* **2019**, *7*, 2419–2426. [CrossRef]
94. Koyuncu, M.A.; Dilmaçünal, T. Determination of vitamin C and organic acid changes in strawberry by HPLC during cold storage. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2010**, *38*, 95–98.
95. Mukherjee, A.; Gómez-Sala, B.; O'connor, E.M.; Kenny, J.G.; Cotter, P.D. Global regulatory frameworks for fermented foods: A review. *Front. Nutr.* **2022**, *9*, 902642. [CrossRef] [PubMed]
96. Pilipenko, V.I.; Isakov, V.A.; Morozov, S.V.; Vlasova, A.V.; Kochetkova, A.A. Efficacy of newly developed kombucha-based specialized food product for treatment of constipation-predominant irritable bowel syndrome. *Vopr. Pitan.* **2022**, *91*, 95–104.