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# Innovative Development of Instant Kombucha Drink and Kombucha Ice Cream Using Spray Drying Techniques

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**ABSTRACT**

This research focuses on the development of instant kombucha drink and kombucha ice cream using spray drying techniques. Kombucha, a fermented tea known for its health benefits, is processed to halt alcohol production and enhance its usability in various forms. The study utilized three treatment methods to ferment kombucha: Cold shock at ≤ 4°C for 5 days, pasteurization at 72°C for 20 mins and yeast removal using a coffee filter, alongside a standard fermentation control. The spray drying process was applied to kombucha from these treatments to produce instant kombucha powder. The physicochemical properties, nutritional quality and microbial safety of the resulting powder were evaluated. The best results were obtained from pasteurization, yielding a powder with low alcohol content (0.13 % w/w), desirable physicochemical properties (4.70 total dissolved solids, 3.10 pH, 0.2177 titratable acidity) and microbial safety (< 1 CFU/ml). Subsequently, instant kombucha powder was utilized to develop two new products: Instant kombucha drink and kombucha ice cream. The instant drink reconstituted well with water, maintaining the original kombucha's flavour profile and health benefits. The kombucha ice cream was formulated to preserve the bioactive compounds and probiotics, offering a novel health-promoting dessert option. Sensory evaluations indicated high consumer acceptance for both products, with the instant drink and ice cream scoring well on taste, texture and overall satisfaction. The innovative use of spray drying to produce instant kombucha powder and its application in diverse products highlights the potential for expanding kombucha's market presence while retaining its health benefits.

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## 1. Introduction

Kombucha, a fermented tea beverage with origins dating back to ancient China, has garnered increasing popularity globally due to its unique flavour profile and perceived health benefits. Traditionally, kombucha is produced by fermenting sweetened black or green tea with a symbiotic culture of bacteria and yeast (SCOBY). This fermentation process results in the production of various bioactive compounds, including organic acids (such as acetic acid, gluconic acid and lactic acid), ethanol, glucuronic acid, phenolic compounds, B vitamins and enzymes [1]. These components contribute not only to kombucha's characteristic tangy taste but also to its potential antioxidant, antimicrobial and detoxifying properties [2].

However, the fermentation process inherent to kombucha production also leads to the formation of alcohol, which has raised concerns regarding product safety and regulatory compliance, particularly in contexts where strict alcohol limits apply, such as halal dietary standards [3,4]. The alcohol content in kombucha can vary widely depending on factors such as fermentation time, temperature, yeast activity and sugar concentration, making consistent product quality and regulatory compliance challenging [5].

Research efforts have focused on developing strategies to mitigate alcohol production while preserving the sensory and nutritional qualities of kombucha. Methods such as cold shock treatment, which reduces yeast activity post-fermentation and pasteurization, which halts microbial activity including yeast and bacteria, have been explored to stabilize alcohol content [6,7]. Additionally, techniques like yeast removal and sugar limitation during fermentation have shown promise in controlling alcohol levels and improving product consistency [8].

The popularity of kombucha has spurred innovation in its production and application. One notable advancement is the development of instant powdered kombucha using spray drying technology. This method not only stabilizes alcohol content but also extends shelf life, making kombucha more accessible and suitable for diverse consumer preferences and market demands [9]. Instant kombucha powder offers convenience in storage, transportation and preparation, while retaining the beverage's nutritional benefits and probiotic properties [10].

Despite its ancient origins, kombucha continues to evolve as researchers explore its bioactive compounds, health benefits and potential applications in functional foods and beverages. Studies have highlighted its antioxidant properties derived from polyphenols in tea, which may contribute to reducing oxidative stress and inflammation [1,11]. Moreover, the probiotic potential of kombucha, attributed to live microbial cultures in SCOBY, underscores its role in gut health and digestive wellness [12].

Kombucha represents a dynamic area of research and development, balancing tradition with innovation to meet modern consumer expectations for health, taste and convenience. As interest in functional foods grows, kombucha's versatility and potential applications continue to expand, driven by ongoing scientific inquiry and technological advancements in fermentation and food processing.

This study addresses the pressing need to develop effective strategies for reducing alcohol production during kombucha fermentation while exploring the potential of instant kombucha powder for innovative applications, such as kombucha ice cream—a product not currently available in the market. The variability in alcohol levels in traditionally fermented kombucha remains a significant concern for regulatory bodies and consumers alike, particularly in regions where adherence to halal dietary laws is crucial. By investigating methods to halt alcohol production, assessing the physicochemical properties, evaluating sensory attributes through hedonic testing and developing instant kombucha powder using advanced spray drying techniques, this study aims to contribute valuable insights into enhancing the safety, quality and market potential of kombucha

products. These objectives were designed to provide a comprehensive understanding of fermentation dynamics, product stability and innovative applications in the context of emerging consumer preferences and regulatory requirements.

## 2. Materials

The black tea utilized in this study was sourced from Releaf Kombucha, based in Penang, Malaysia. Sucrose was obtained from Gula Prai, produced by MSM Prai Berhad in Seberang Prai, Pulau Pinang. SCOBY and starter tea were supplied by Saintifik Kinabalu Sdn Bhd. Maltodextrin with a DE value of 10-12 was procured from Qinhuangdao Lihua Starch Co., LTD in China. All remaining chemicals used were of analytical grade.

### *2.1 Experimental Design*

This study was conducted in three phases, tea preparation that dealing with different concentration of sugar at 9, 6 and 3 %; duration of fermentation involves 7 and 14 days; special treatment (cold shock, pasteurization and filtration of yeast removal) to reduce alcohol production. In the initial phase, kombucha samples were prepared, according to Table 1 below.

**Table 1**
Kombucha formulation

| Ingredients | Control | Formulation 1 | Formulation 2 |
|---|---|---|---|
| Black tea bags | 3 | 3 | 3 |
| Water (mL) | 1000 | 1000 | 1000 |
| Sugar (%) | 9 | 6 | 3 |
| SCOBY | 1 | 1 | 1 |

***Note:*** SCOBY (symbiotic culture of bacteria and yeast)

Meanwhile for the second phase, the kombucha samples will differentiate according to the duration of fermentation that known as first phase- and second-phase fermentation, for 7- and 14-days, respectively. All the testing parameters involving day 0, 7 and 14 considering fermentation duration. During the third phase, kombucha samples has undergone for the treatments comprising one control and three treatments: Treatment one involved subjecting the samples to cold shock; Treatment two entailed pasteurization at 75-85°C for 20 mins; Treatment three focused on removing yeast from the starter liquid of the previous kombucha batch.

The samples were analysed for alcohol content, total soluble solids (TSS), pH and titratable acidity (TA) to assess their physicochemical properties. Proximate analysis included determination of moisture content, carbohydrate content, protein content and ash content. Additionally, Plate Count Method and hedonic tests were conducted. In the last phase, instant kombucha powder was produced through spray drying. The resulting powder was analysed for yield percentage, colour, water activity and solubility of the instant kombucha tea. Figure 1 shows an experimental design flow chart.

#### *2.1.1 Preparation of low alcohol kombucha*

The preparation of low alcohol kombucha involved several steps based on established protocols [13]. Initially, one litre of water was boiled and poured into a glass jar, where 3 tea bags of black tea was steeped for 5 mins. After removing the tea bags, 9, 6 and 3 % sugar was added for the control

and treatments, ensuring complete dissolution (Table 1). The solution was then cooled to room temperature (< 28°C), and the pH was adjusted using starter tea from previous batches to achieve an optimal pH of ≤ 4.5, which is conducive to SCOBY growth.

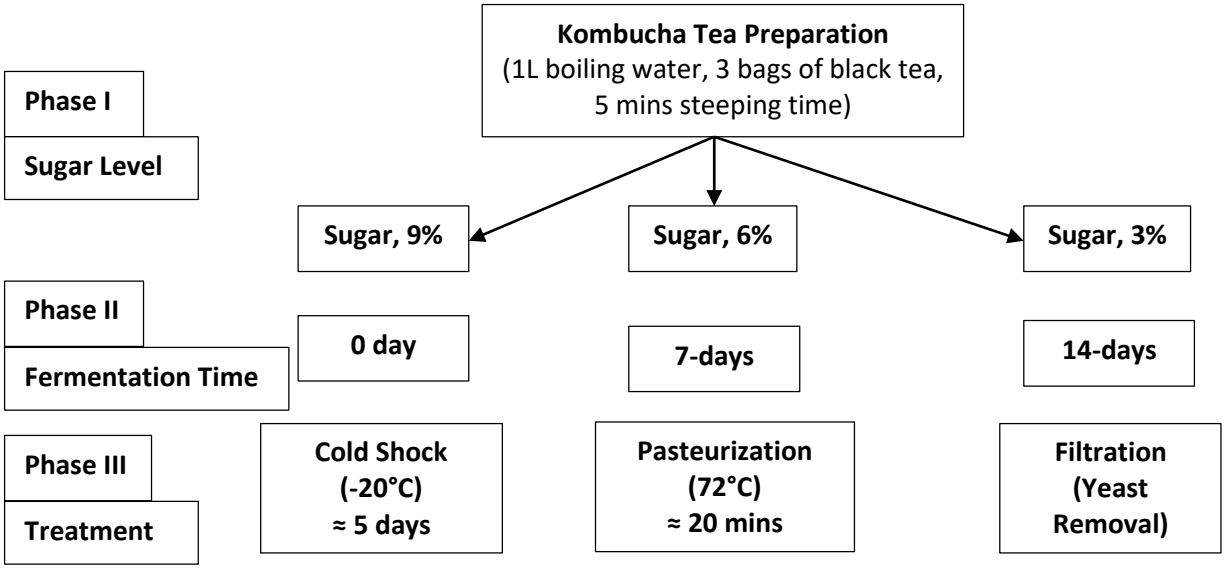


**Fig. 1.** Experimental design flow chart

Next, SCOBY (5 %) was introduced into the solution, and fermentation was allowed to proceed for 7 days at room temperature. Post-fermentation, 75 % of the kombucha was strained and bottled for storage, while the remaining portion (25 %) was retained with SCOBY for subsequent batches. This process was repeated with varied sugar concentrations (9, 6 and 3 %) to assess alcohol production. For treatment one (cold shock), the kombucha underwent freezing temperature at -20°C for five days before further analysis. Treatment two (pasteurization) involved heating the kombucha to 72°C for 20 mins to halt fermentation and reduce alcohol content. In treatment three (yeast removal), the starter tea was filtered before and after fermentation to eliminate yeast cells. Throughout the process, quality control measures were implemented, including periodic sampling for analysis of alcohol content, TSS, pH and TA. These steps ensured the production of low alcohol kombucha suitable for consumers seeking beverages with reduced alcohol content while retaining the characteristic flavours and health benefits associated with traditional kombucha.

### *2.2 Determination of Physicochemical Properties of Kombucha Tea*

#### *2.2.1 Ethanol concentration*

Ethanol concentration was measured using the Headspace-Gas Chromatography-Flame Ionization Detector (GC-FID) method, following [14]. Briefly, 100 mL kombucha sample underwent sonication to remove carbonation, followed by mixing with 5 mL of an internal standard solution (1-propanol) in a 100 mL volumetric flask. After centrifugation and filtration, the sample was prepared for Gas Chromatography (GC) analysis. Ethanol concentration was determined using an external standard calibration curve [15].

### *2.2.2 Total soluble solids (TSS)*

TSS were measured using Brix scale and a portable laboratory refractometer (RL3, Polish Optical Works, Warsaw, Poland) calibrated with distilled water. Drops of kombucha sample were placed on the refractometer prism, and TSS readings were recorded following standard procedures [16].

### *2.2.3 pH*

pH levels of fermented kombucha samples were determined using a pH meter (SCHOTT Instruments; SI Analytics Mainz, Mainz, Germany). About 30 mL of sample was calibrated with pH 4, 7 and 10 buffer solutions. The electrode was immersed in the sample, and readings were taken after stabilization, following methods adapted from [17].

### *2.2.4 Titratable acidity (TA)*

TA was measured by titrating 50 mL of kombucha sample with 0.1 M sodium hydroxide to pH 8.2. TA was calculated as a percentage of acetic acid per gram of sample, a key organic acid in kombucha [18, 19] using the Eq. (1):

$$\%\, Titratable\ acidity\ of\ acetic\ acid = \frac{V_{NaOH} \times M_{NaOH} \times 6.0053}{V_{sample}} \quad (1)$$

## *2.3 Determination of Proximate Content of Kombucha Tea*

### *2.3.1 Moisture content*

An aluminium dish and cover were dried at 105°C for 3 hours, cooled in a desiccator, and weighed. 3 g of kombucha sample was dried in the dish at 105°C, cooled and reweighed to calculate moisture content.

### *2.3.2 Ash content*

A crucible and cover were heated at 550°C overnight, cooled in a desiccator and weighed. 3 g of sample was ashes in the crucible at 550°C, cooled and reweighed to determine ash content.

### *2.3.3 Protein content*

Protein content was determined using the Kjeldahl method. 2 g of sample was digested with concentrated sulfuric acid and selenium tablets at 400°C. After cooling, distilled water was added and distillation with sodium hydroxide/boric acid was performed using Kjeltec™ 2030. Protein content was calculated from the distillation results.

### *2.3.4 Carbohydrate content*

Carbohydrate content was calculated by subtracting moisture, protein, fat and ash content percentages from 100 %.

### *2.4 Determination of Total Phenolic Content*

Total Phenolic Content (TPC) was measured using the Folin-Ciocâlteu method. Kombucha samples were reacted with Folin-Ciocâlteu reagent and sodium carbonate, incubated and absorbance was measured at 760 nm. TPC was calculated using the equation:

$$TPC\ (mg/ml) = \frac{C \times V}{m} \quad (2)$$

where C is the concentration from the calibration curve, V is the sample volume and m is the sample mass.

### *2.5 Determination of Total Flavonoids Content*

Total Flavonoids Content (TFC) was determined using the aluminium chloride colorimetric method. Samples were reacted with aluminium chloride and potassium acetate, incubated and absorbance was measured at 415 nm. TFC was calculated using the equation:

$$TFC\ (mg/ml) = \frac{C \times V}{m} \quad (3)$$

where C is the concentration from the calibration curve, V is the sample volume and m is the sample mass.

### *2.6 Antioxidant Activity by the DPPH Method*

Antioxidant activity was assessed using the DPPH (2.2-Diphenyl-1-Picrylhydrazyl) method. Samples were mixed with DPPH solution, incubated in the dark and absorbance was measured at 517 nm. Antioxidant activity was calculated as:

$$\%\ \text{Antioxidant activity} = \frac{Absorance\ of\ the\ control - absorbance\ of\ the\ test\ sample}{Absorbance\ of\ the\ control} \times 100\% \quad (4)$$

### *2.7 Microbiological Test*
### *2.7.1 Growth media*

Potato Dextrose Agar (PDA) was prepared at 39.0 g per 1000 mL, sterilized at 121°C for 20-25 mins, cooled to 50°C and poured into Petri dishes. After solidification, dishes were incubated inverted [20].

### *2.7.2 Colony count*

Colony counting for molds and yeast was conducted after 2 days of incubation. Plates with 10-150 colonies were averaged for colony-forming units (CFU)/g or CFU/mL, rounded to two significant figures [21].

### *2.7.3 Estimation of yeast and mold count*

Yeast and mold count per millilitre were estimated using the Bureau of Indian Standard [22] formula, rounding to two significant figures. Results < 1 x 10^$^{-1}$ CFU/mL were reported as less than 1.

### *2.7.4 Determination of acetic and lactic acid bacteria*

Acetic acid bacteria (AAB) and lactic acid bacteria (LAB) were isolated using serial dilutions and specific media. AAB and yeast were incubated at 30 and 25°C respectively, while LAB were incubated anaerobically at 37°C. CFU were counted and expressed per mL or g. Representative AAB were purified on potato dextrose agar (PDA).

### *2.8 Sensory Evaluation*

Sensory evaluation of kombucha samples was conducted using acceptance testing in an affective test with 50 untrained panellists. Panellists were asked to score the overall acceptance difference between various samples. All kombucha samples were presented simultaneously. In this process, participants evaluated the overall acceptance of the sample using a 9-point hedonic scale [23]. Mean scores were calculated to indicate sensory quality. Sample servings of kombucha were 15 mL, that coded 279, 342, 945, 265, 662 and 479 according to permutation table [24].

### *2.9 Instant Preparation of Kombucha Tea*

The spray-drying process was conducted using a spray-drying machine where the inlet temperature was 160°C, outlet temperature 80°C, with 20.0 rpm and maltodextrin as a binding agent for the kombucha tea, 8 - 10 % of the total volume of kombucha tea to be dried.

### *2.9.1 Kombucha powder analysis*
#### *2.9.1.1 Yield percentage*

The yield percentage of kombucha powder was calculated using the formula as mentioned by [25].

$$\%yield = \left(\frac{Total\ weight\ of\ powder\ solid}{total\ weight\ of\ solid\ in\ liquid\ sample}\right) \times 100 \quad (5)$$

#### *2.9.1.2 Water activity*

The water activity of kombucha powder was measured using a Rotronic Hygrolab instrument. A 3-gram sample was placed in the instrument, and the water activity value was recorded [26].

#### *2.9.1.3 Solubility*

Solubility was determined by adding 0.5 grams of kombucha powder to 50 mL of distilled water, mixing and centrifuging [27,28]. The supernatant was dried, and the solubility percentage was calculated as:

$$\%Solubility = \left(\frac{weight\ difference\ after\ \ drying}{0.5g}\right) \times 100 \quad (6)$$

*2.9.1.4 Colour*

Colour analysis of kombucha powder was conducted using a calibrated Hunter Lab Colorflex Colorimeter. The L*, a*, b* values were measured, and the total colour difference (ΔE*) was calculated if significant.

$$\Delta E* = \sqrt{(\Delta a*)^2 + (\Delta b*)^2 + (\Delta L*)^2} \quad (7)$$

*2.10 Statistical Analysis*

Statistical analysis was conducted using IBM SPSS version 29. The data obtained from physicochemical analysis, proximate analysis of kombucha tea, microbiological analysis, sensory evaluation analysis and kombucha powder analysis were analysed using one-way ANOVA presented in the form of Mean ± SD. Followed by Tukey's HSD test if there was a significant difference between mean values ($p > 0.05$), and the results were presented as Mean ± SD.

## 3. Results and Discussion

*3.1 Treatment Methods*

Sugar levels and fermentation duration are well known to affect both alcohol production and the taste of kombucha [29-34]. Therefore, this study focused on three different sugar levels (9, 6 and 3 %) and two fermentation periods (7 and 14 days).

Cold shock, pasteurization and yeast removal methods were chosen for use in the kombucha production process due to their high likelihood of effectively reducing alcohol production in this beverage, as indicated by previous research. Cold shock, for instance, involves rapid exposure of kombucha to low temperatures after the main fermentation process. This helps abruptly stop yeast and bacterial activity, thereby reducing alcohol production.

Pasteurization involves heating kombucha to kill microorganisms, including yeast, involved in fermentation. This process helps ensure better control over alcohol content. Meanwhile, yeast removal method involves separating and removing yeast from kombucha after fermentation. This ensures that potentially alcohol-producing yeast are eliminated, thereby reducing the risk of high alcohol content in the beverage. In this study, pasteurization treatment successfully produced the lowest alcohol content at 0.13 % w/w. This is because pasteurization kills cultures and prevents the continuous formation of carbon dioxide or alcohol in the bottle [35].

*3.2 Physicochemical Analysis*

*3.2.1 pH value*

pH is a fundamental parameter that influences the quality, safety and flavour of kombucha. It measures the acidity or alkalinity of a solution on a scale from 0 to 14, with 7 being neutral. In kombucha fermentation, pH significantly affects microbial activities, the fermentation process, taste and preservation.

Table 2 shows the pH values of all formulations decreased over time, with the control (9 % sugar) showing a more significant drop compared to formulations with 6 and 3 % sugar. By day 14, the pH levels were lower across all formulations, indicating increased acidity due to the fermentation

process. The Levene statistic test ($p$ = 0.021) indicated significant differences among the formulations, suggesting non-homogeneous variances ($p$ < 0.05). The ANOVA results confirmed significant differences in pH values between different formulations ($p$ < 0.001). These findings suggest that higher sugar content accelerates fermentation, resulting in a quicker pH decline and higher acidity. This is likely due to increased production of organic acids such as acetic acid during fermentation.

**Table 2**
Average pH values of different formulations of kombucha over fermentation time

| Time (days) | Control (9 % sugar) | 6 % Sugar | 3 % Sugar |
|---|---|---|---|
| Day 0 | $5.90 \pm 0.07^{aA}$ | $5.63 \pm 0.07^{aB}$ | $5.52 \pm 0.11^{aC}$ |
| Day 7 | $2.96 \pm 0.01^{bA}$ | $2.90 \pm 0.02^{bA}$ | $2.86 \pm 0.01^{bB}$ |
| Day 14 | $2.85 \pm 0.01^{cA}$ | $2.77 \pm 0.02^{cB}$ | $2.75 \pm 0.02^{cB}$ |

***Note:*** Values with different superscripts within a row are significantly different ($p$ < 0.05). Values with different superscripts within a column are significantly different ($p$ < 0.05)

### *3.2.2 Sugar concentration*

Sugar concentration is crucial in kombucha production, serving as the primary energy source for the SCOBY. Yeast converts sugar into alcohol and carbon dioxide, which bacteria then metabolize into organic acids. Table 3 shows sugar concentrations decreased across all formulations over the fermentation period, with the highest decrease observed in the control formulation by day 14. The ANOVA ($p$ = 0.469) and homogeneity ($p$ = 0.345) tests indicated no significant differences in sugar concentrations between formulations ($p$ > 0.05). During fermentation, sugar is hydrolysed into glucose and fructose by yeast, which is then metabolized to produce ethanol. Over time, sugar content decreases slightly, reflecting the fermentation process.

**Table 3**
Sugar concentration of different formulations of kombucha over fermentation time

| Time (days) | Control (9 %) | Sugar (6 %) | Sugar (3 %) |
|---|---|---|---|
| Day 0 | $10.00 \pm 1.00^{aA}$ | $9.67 \pm 0.30^{aB}$ | $10.00 \pm 0.00^{aA}$ |
| Day 7 | $9.33 \pm 0.60^{bA}$ | $9.33 \pm 0.30^{abA}$ | $9.00 \pm 0.50^{bB}$ |
| Day 14 | $9.00 \pm 1.00^{cA}$ | $8.93 \pm 0.40^{bA}$ | $9.00 \pm 0.50^{bA}$ |

***Note:*** Values with different superscripts within a row are significantly different ($p$ < 0.05). Values with different superscripts within a column are significantly different ($p$ < 0.05)

### *3.3 Titratable Acidity*

Titratable acidity measures the total amount of acid in kombucha, which influences its taste and stability. It reflects the concentration of organic acids produced during fermentation. Table 4 presents the mean titratable acidity values along with their standard deviations for each formulation of kombucha at different fermentation times. Titratable acidity increased across all formulations, with the control showing the highest acidity by day 14. The increase in titratable acidity is due to the ongoing microbial activity and production of organic acids like acetic acid during fermentation.

The control formulation showed the highest acidity, aligning with its lower pH values. The ANOVA results confirmed significant differences in titratable acidity between different formulations ($p$ < 0.001).

**Table 4**
Titratable acidity of different formulations of kombucha over fermentation time

| Fermentation Time | Control (g/L) | 6 % Formulation (g/L) | 3 % Formulation (g/L) |
|---|---|---|---|
| Day 0 | $1.80 \pm 0.20^{cA}$ | $1.67 \pm 0.12^{bB}$ | $1.53 \pm 0.45^{cBC}$ |
| Day 7 | $3.43 \pm 0.06^{bA}$ | $3.47 \pm 0.12^{aA}$ | $2.50 \pm 0.00^{bB}$ |
| Day 14 | $4.30 \pm 0.17^{aA}$ | $3.80 \pm 0.17^{aB}$ | $3.87 \pm 0.12^{aB}$ |

***Note:*** Values with different superscripts within a row are significantly different ($p < 0.05$). Values with different superscripts within a column are significantly different ($p < 0.05$)

### *3.3.1 Ethanol concentration*

Ethanol in kombucha results from yeast converting sugars into alcohol and carbon dioxide. The symbiotic activity of bacteria and yeast particularly *Saccharomyces cerevisiae*, produces ethanol which AAB subsequently convert into acetic acid, contributing to kombucha's tangy flavour.

**Table 5**
Ethanol concentration in kombucha formulations over fermentation time

| Formulations | Ethanol concentration (% w/w) |
|---|---|
| Control (9 %) Day 7 | $0.18^{a}$ |
| Formulation 1 (3 %) Day 7 | $0.22^{a}$ |
| Formulation 2 (6 %) Day 7 | $0.05^{b}$ |
| Control (9 %) Day 14 | $0.02^{b}$ |
| Formulation 1 (3 % Day 14 | $0.06^{b}$ |
| Formulation 2 (6 %) Day 14 | $0.13^{a}$ |

***Note:*** Values with different superscripts within a row (between formulation and the same day of fermentation) are significantly different ($p < 0.05$)

As shown in Table 5, ethanol concentration increased during the first 7 days of fermentation, peaking between 0.05-0.22 %, and then declined by Day 14 to 0.02-0.13 %. This pattern aligns with Jakubczyk *et al*., [36], indicating maximum alcohol production on the 7$^{th}$ day followed by a decrease due to microbial activity converting ethanol to other compounds. Jakubczyk *et al*., [36] demonstrated that ethanol concentration peaks at around 3.0-3.5 % on the 7$^{th}$ day of fermentation, aligning with our observed pattern of ethanol increase followed by a decline.

In the third phase, alcohol production in kombucha was successfully reduced using the aforementioned treatments. Comparing with alcohol analysis of original kombucha studies before this by [37-39] with ethanol analysis results of 0.94 per weight, 0.32 % for 7$^{th}$ day fermentation period, and 0.7 - 1.3 % range of alcohol content in kombucha obtained from results of investigations by Food and Drug Administration (FDA), percentage analysis of alcohol content of each sample treated with different methods as stated based on Table 6 were the lowest. When the alcohol control is 0.25 %, cold (R1) shock, pasteurization (R2) 0.13 % and yeast removal (R3) 0.15 % reductions occurs and the alcohol content was observed.

**Table 6**
Ethanol concentration of kombucha at different treatment

| Sample / Treatment | Ethanol concentration (% w/w) |
|---|---|
| K | $0.25^{a}$ |
| R1 | $0.18^{b}$ |
| R2 | $0.13^{bc}$ |
| R3 | $0.15^{bc}$ |

***Note:*** K = control, R1 = cold shock, R2 = pasteurization, and R3 = yeast removal. Values are expressed as mean ± standard deviation except for percentage of ethanol. Values with different superscripts within a row are significantly different ($p < 0.05$)

### *3.4 Proximate Analysis*

#### *3.4.1 Moisture content*

Moisture content is the measurement of total water present in a food product, usually expressed as a percentage by weight on a wet basis. To prevent microbial growth, moisture content needs to be maintained below approximately 10 %, depending on the type of food [40]. According to Table 7, the moisture content percentage of control sample (K) shows significant differences ($p < 0.05$) compared to values of cold shock treatment (R1), pasteurization treatment (R2) and filtration or yeast removal treatment (R3). K has a lower water content of 91.6120 ± 0.2947, compared to R1 95.7191 ± 0.7084, R2 95.0568 ± 0.5289 and R3 95.1808 ± 0.0722. The lower moisture percentage in K indicates higher dissolved solids content, resulting in lower total water content in K. When compared to the standard moisture content, all kombucha samples have high moisture percentages, exceeding 10 % and tend towards microbial contamination.

#### *3.4.2 Protein content*

Based on Table 7, the analysis results of protein percentage for K, R1, R2 and R3 show no significant differences ($p > 0.05$). All kombucha samples show negative protein percentage values, except K which is 0.0028 ± 0.0330. This indicates that the protein content in kombucha fermented for seven days is low or negligible. According to Martínez-Leal *et al.,* [41], tea contains various amino acids and proteins. The increase in amino acid content correlates directly with fermentation time, as yeast and bacteria break down tea components and sugars during fermentation. Also, according to Tejedor-Calvo & Morales [42], protein content in conventional kombucha is very low compared to other macronutrients, typically around 3 µg/mL in conventional and commercial tea kombucha. The low and negative protein analysis percentages of samples K, R1, R2 and R3 may be due to the short fermentation period of seven days, resulting in amino acid or protein components not being produced or being too low to be detected by the protein analysis machine.

#### *3.4.3 Ash content*

Ash refers to non-organic residue remaining after complete combustion or oxidation of organic matter in a food product. Ash content represents the total mineral amount in food [43]. According to Table 7, the ash content percentage of K at 0.3442 ± 0.0123, which is higher, shows significant differences ($p < 0.05$) compared to ash content percentage values of R1, R2 and R3. This indicates that sample K has a higher total mineral content. The difference in ash percentage results may be due to the preparation process of kombucha, where tea is steeped in hot water, allowing mineral extraction from tea leaves into the liquid. If the tea is removed too early, mineral values may not be fully extracted. Minerals extracted from tea leaves contribute to the overall mineral content, including ash content, in the final kombucha product. Also, during filtration to remove solid particles, dissolved minerals contributing to ash content may also be removed.

#### *3.4.4 Carbohydrate content*

Based on Table 7, the carbohydrate content percentage of K is significantly different ($p < 0.05$) compared to F1, F2 and F3. K had the highest carbohydrate percentage of 8.0543 ± 0.2345, followed by R2, R3 and R1. This substantial difference was due to the high sugar content in K. Sugar (sucrose) is a carbohydrate [44], contributing to the percentage carbohydrate value. According to Tejedor-

Calvo & Morales [42], prepared kombucha shows varying levels of carbohydrate content. This variation was due to the specific SCOBY used, as different SCOBY concentrations provide different carbohydrate concentrations.

**Table 7**
Proximate compositions of kombucha undergone different treatment

| Sample | Moisture, % | Protein, % | Ash, % | Carbohydrate, % |
|---|---|---|---|---|
| K | 91.6120 ± 0.2947[a] | 0.0028 ± 0.0330[a] | 0.3442 ± 0.0123[b] | 8.0543 ± 0.2345[b] |
| R1 | 95.7191 ± 0.7084[b] | -0.0175 ± 0.0111[a] | 0.2387 ± 0.0316[a] | 4.0596 ± 0.7043[a] |
| R2 | 95.0568 ± 0.5289[b] | -0.0179 ± 0.0108[a] | 0.2469 ± 0.0189[a] | 4.7142 ± 0.5208[a] |
| R3 | 95.1808 ± 0.0722[b] | -0.0145 ± 0.0137[a] | 0.2405 ± 0.0435[a] | 4.5661 ± 0.0112[a] |

***Note:*** K = control, R1 = cold shock, R2 = pasteurization and R3 = yeast removal. Values are expressed as mean ± standard deviation. Values with different superscripts within a row are significantly different ($p < 0.05$)

### *3.5 Microbial Analysis*

Human errors typically cause toxic effects in kombucha through harmful biological and chemical biology as well as excessive consumption. Harmful biological concerns associated with kombucha intake include food pathogens such as *Salmonella spp., Listeria monocytogenes, Bacillus spp., Staphylococcus aureus* and *Clostridium botulinum*. Contamination by molds with organisms such as *Penicillium* and *Aspergillus* can also occur in kombucha. These microorganisms produce mycotoxins associated with carcinogenic and aflatoxicosis effects in humans. These biological hazards are usually linked to poor hygiene practices [45].

Microbial testing was conducted twice for three treatment groups. In the first batch, all three groups showed TNTC (Too Numerous to Count) for dilutions $10^{-1}$ - $10^{-4}$. Meanwhile in the second batch of kombucha, besides yeast, mold presence was also observed. However, the mold was not considered because according to [21], mold plate counting was only performed if it contains 10 - 150 colonies. Microbial analysis of kombucha was performed up to seven dilutions due to the turbidity of the kombucha liquid. According to Tran *et al.,* [46], turbid water contains $>10^7$ bacteria per millilitre. Based on the microbial study results, samples K, R1, R2 and R3 still showed CFU/ml observed at the $7^{th}$ dilution.

This microbial analysis was conducted to determine whether kombucha samples were exposed to mold contamination and to determine the CFU/ml count, including yeast contained in the kombucha samples before serving to the sensory panel for hedonic evaluation purposes. Table 8 shows the results of microbial analysis in terms of estimated counts of yeast and mold per millilitre. Sample K had the highest estimated CFU/ml value over seven dilutions compared to R3 and R1. Meanwhile, no CFU/ml presence was observed in R2, which was pasteurized at 72°C for 20 mins. As noted by Jayabalan *et al.,* [1], yeast and bacteria will die at temperatures > 50°C.

According to Regulation 39 concerning Microbiological Standards in the Food Regulations 1985, the maximum limit of plate counts shall be equal to or less than 10^5/ml for food and beverages ready for consumption. However, it is unclear whether this statement can be applied to the category of ready-to-drink beverages or not because there is no specific Codex standard for CFU/ml standards for kombucha beverages at this time [13].

**Table 8**
Estimated average CFU/ml in seven dilutions of second batch of kombucha

| Sample | CFU/ml |
|---|---|
| K | $1.8 \times 10^7$ |
| R1 | $2.3 \times 10^6$ |
| R2 | < 1 |
| R3 | $1.6 \times 10^7$ |

***Note:*** K = control, R1 = cold shock, R2 = pasteurization and R3 = yeast removal

### *3.5.1 Acetic acid bacteria cell counts*

Acetic acid bacteria (AAB) play a crucial role in kombucha fermentation, converting ethanol into acetic acid. The concentration and viability of AAB can vary across formulations and fermentation times. Table 9 shows the viable counts of AAB generally increased over time, particularly in the control formulation. Pasteurized samples showed variable counts, indicating possible issues in the pasteurization process. The ANOVA results confirmed significant differences in AAB cell counts between different formulations ($p < 0.001$). The viable counts of AAB generally increased over time, with higher counts observed in the control formulation. This trend is likely due to the higher sugar content, which provides more substrate for microbial growth.

**Table 9**
Acetic acid bacteria cell counts (x $10^3$ CFU/ml) of different formulations of kombucha over fermentation time

| Time (days) | Control | Control (*P*) | Formulation 1 | Formulation 1 (*P*) | Formulation 2 | Formulation 2 (*P*) |
|---|---|---|---|---|---|---|
| Day 7 | $0.60 \pm 0.3^{bB}$ | $1.55 \pm 0.6^{aA}$ | $1.25 \pm 0.2^{aA}$ | $0.95 \pm 0.3^{bB}$ | $1.4 \pm 0.6^{aA}$ | $1.1 \pm 0.0^{bB}$ |
| Day 14 | $2.35 \pm 0.3^{aA}$ | $1.15 \pm 0.1^{bB}$ | $0.85 \pm 0.3^{bB}$ | $1.05 \pm 0.2^{aA}$ | $1.2 \pm 0.4^{bB}$ | $1.5 \pm 0.3^{aA}$ |

***Note*:** (P) – Pasteurized. Values with different superscripts within a row are significantly different ($p < 0.05$). Values with different superscripts within a column (between formulation) are significantly different ($p < 0.05$)

### *3.6 Phytochemical Contents of Kombucha*
### *3.6.1 Total phenolic content (TPC)*

Polyphenols in tea are primarily responsible for the antioxidant activity of kombucha. This study corroborates Chakravorty *et al.,* [2], noting an increase in polyphenols including flavonoids during fermentation. Additionally, thearubigin transforms into theaflavin, resulting in a colour change in kombucha from dark to light as fermentation progresses.

**Table 10**
Concentration of GAE and TPC in kombucha formulations over fermentation time

| Formulations | GAE (mg/ml) | TPC (mg GAE/ml) |
|---|---|---|
| Control (9 %) Day 7 | 0.5332 | $17.78 \pm 1.80^b$ |
| Formulation 1 (3 %) Day 7 | 0.4052 | $13.51 \pm 1.90^b$ |
| Formulation 2 (6 %) Day 7 | 0.4342 | $14.48 \pm 2.00^b$ |
| Control (9 %) Day 14 | 0.5502 | $18.34 \pm 0.30^a$ |
| Formulation 1 (3 %) Day 14 | 0.4712 | $15.70 \pm 1.10^a$ |
| Formulation 2 (6 %) Day 14 | 0.5892 | $16.58 \pm 0.70^a$ |

***Note:*** GAE = Gallic acid equivalents, TPC = Total phenolic content. Values with different superscripts within a row (between formulation and the same day of fermentation) are significantly different ($p < 0.05$)

Table 10 shows an increase in TPC over fermentation time. Specifically, the control formulation increased from 17.78 - 18.34 mg GAE/ml from Day 7 - Day 14. Similarly, Formulation 1 and Formulation 2 showed increases in TPC, indicating that fermentation time influences the rise in polyphenols. Kombucha prepared from green and black tea exhibited an increase in polyphenolic compounds, peaking on the 14$^{th}$ day due to enzymatic oxidation during fermentation. This aligns with Jayabalan *et al*., [1], who observed similar polyphenol increases due to microbial hydrolysis reactions. The rise in polyphenolic compounds observed during the fermentation process is consistent with findings by [1], who also reported an increase due to enzymatic oxidation reactions during fermentation. The statistical analysis shows no significant differences in TPC among different kombucha formulations ($p > 0.05$). Suhardini and Zubaidah [47] explained that microbial involvement in fermentation produces phenolic compounds, but increases are not always significant.

### *3.6.2 Total flavonoid content (TFC)*

Flavonoids are secondary metabolites in plants, acting as antioxidants by donating electrons to inhibit oxidation. The standard calibration curve for quercetin determined flavonoid concentration in kombucha. Table 11 shows the control formulation had the highest flavonoid content on Day 7, while Formulation 1 had the lowest. This fluctuation may result from flavonoid degradation or microbial activity during fermentation, which affects stability and concentration. However, microbial action can also increase flavonoid levels by degrading polyphenols into flavonoids [48]. This study supports [2], who noted an increase in polyphenols during fermentation, including flavonoids, with thearubigin transforming into theaflavin, resulting in colour changes in kombucha. The variations in flavonoid content during fermentation can be attributed to microbial activity, as discussed by [48], who found that microorganisms such as *Lactobacillus plantarum* can degrade polyphenols into flavonoids.

The statistical analysis indicates a significant difference in flavonoid content among formulations ($p$ = 0.031). However, ANOVA shows no significant differences ($p$ = 0.663), meaning the null hypothesis cannot be rejected. La Torre *et al.,* [49] also observed a decrease in flavonoid content during fermentation, remaining constant over time. They noted that flavonoid levels can be affected by microbial activity and fermentation conditions, with possible degradation over time.

**Table 11**
Quercetin equivalents and total flavonoid content in kombucha formulations over fermentation time

| Formulations | Quercetin Equivalents (mg/mL) | Total Flavonoid Content (mg GAE/mL) |
|---|---|---|
| Control (9 %) Day 7 | 6.4512 | $0.65 \pm 0.004^{a}$ |
| Formulation 1 (3 %) Day 7 | 5.0464 | $0.50 \pm 0.072^{a}$ |
| Formulation 2 (6 %) Day 7 | 5.7091 | $0.57 \pm 0.015^{a}$ |
| Control (9 %) Day 14 | 5.2091 | $0.52 \pm 0.080^{b}$ |
| Formulation 1 (3 %) Day 14 | 5.0067 | $0.58 \pm 0.080^{a}$ |
| Formulation 2 (6 %) Day 14 | 5.3837 | $0.53 \pm 0.073^{a}$ |

***Note:*** GAE = Gallic acid equivalents, TPC = Total phenolic content. Values with different superscripts within a row (between formulation and the same day of fermentation) are significantly different ($p < 0.05$)

### *3.7 Antioxidant DPPH Assay*

The DPPH assay measures the antioxidant potential of compounds in kombucha by assessing the ability of antioxidants to neutralize free radicals. Kombucha contains bioactive compounds like polyphenols, flavonoids and vitamins, known for their antioxidant properties. The DPPH assay

quantifies and compares antioxidant content among different formulations or fermentation times of kombucha, helping understand how fermentation affects antioxidant production or degradation.

Table 12 shows an increase in antioxidant activity from day 7 – 14$^{th}$ for the control formulation (0.376 - 0.417). However, both F1 and F2 showed a decrease in antioxidant activity from day 7 to day 14. This suggests that while fermentation initially boosts antioxidant properties, prolonged fermentation might lead to a decrease. The tests of homogeneity of variances show a significant difference between antioxidant DPPH assay and different kombucha formulations ($p < 0.05$, 0.001). However, the ANOVA results show no significant differences in antioxidant activity between formulations ($p > 0.05$, 0.214), indicating that the null hypothesis cannot be rejected. The lack of significant differences could be due to several reasons, such as antioxidant equilibrium where activity might have reached saturation, microbial adaptation to varying sugar concentrations, or the sensitivity of the DPPH assay not being sufficient to detect subtle differences.

**Table 12**
Concentration of control sample and DPPH assay of different formulations of kombucha and fermentation time

| Formulation | Control Sample (μm/L) | DPPH Assay (μm/L) |
|---|---|---|
| Control (9 %) Day 7 | 0.506 | 0.376$^{b}$ |
| Formulation 1 (3 %) Day 7 | 0.506 | 0.377$^{a}$ |
| Formulation 2 (6 %) Day 7 | 0.506 | 0.459$^{a}$ |
| Control (9 %) Day 14 | 0.506 | 0.417$^{a}$ |
| Formulation 1 (3 %) Day 14 | 0.506 | 0.376$^{a}$ |
| Formulation 2 (6 %) Day 14 | 0.506 | 0.437$^{a}$ |

***Note:*** Values with different superscripts within a row (between formulation and the same day of fermentation) are significantly different ($p < 0.05$)

### *3.8 Sensory Hedonic Test*

#### *3.8.1 Organoleptic properties of different formulation of kombucha*

Organoleptic properties refer to the sensory characteristics of a substance, such as appearance, aroma, taste and mouthfeel. Evaluating these properties in kombucha is crucial for understanding its sensory attributes and overall quality, as they significantly influence consumer preferences and acceptance. The beverage's aroma, taste profile, colour and visual appeal impact whether consumers enjoy and repurchase the product. Understanding these sensory attributes helps in formulating kombucha that aligns with consumer preferences.

The Levene statistics test show differences in consumer acceptance of various kombucha formulations based on six attributes: colour (1.583), aroma (2.948), sweet flavour (2.053), sour flavour (1.714), mouthfeel (fizziness) (2.521) and overall acceptance (1.629). Most panellists preferred the 14$^{th}$ day control formulation for its colour, aroma and sweet flavour compared to other formulations. However, the 14$^{th}$ day F2 formulation was most preferred for sour flavour and mouthfeel (fizziness).

The tests of homogeneity of variances (Levene statistic) show no significant differences in the colour of kombucha formulations ($p > 0.05$, 0.165), indicating equivalent variances. However, aroma ($p < 0.05$, 0.013) and mouthfeel ($p < 0.05$, 0.030) show significant differences, suggesting no homogeneity of variances between formulations. Sweet flavour, sour flavour and overall acceptance show no significant differences, indicating homogeneous variances ($p > 0.05$).

Through ANOVA tests, no significant differences in colour ($p = 0.532$), aroma ($p = 0.097$) and mouthfeel (fizziness) ($p = 0.122$) between formulations ($p > 0.05$). However, there are significant differences in sweet flavour ($p = 0.004$), sour flavour ($p = 0.012$) and overall acceptance ($p = 0.016$),

which are ($p$ < 0.05), leading to rejection of the null hypothesis. Post hoc tests reveal significant differences in sweet flavour between day 7 F1 and day 14 control ($p$ = 0.002), sour flavour between day 14 F1 and day 14 F2 ($p$ = 0.013), and overall acceptance between day 7 formulation and 14$^{th}$ day control and F2 ($p$ < 0.05).

From the homogeneous subsets, there is no significant differences in colour, aroma and mouthfeel among formulations as they are in the same subset. Significant differences in sweet flavour, sour flavour and overall acceptance are noted, with panellists preferring the 14$^{th}$ day control formulation for sweet flavour and F2 for sour flavour and overall acceptance. The panellists rated the 14$^{th}$ day control and F2 formulations moderately high on the hedonic scale.

Thus, significant differences in organoleptic properties among kombucha formulations may arise from varying ingredients, microbial composition or pH/acidity levels. The absence of significant differences could stem from minor variations, panellists' sensitivity or similarities in key constituents among formulations.

### *3.8.2 Organoleptic properties of different treatment of kombucha*

#### *3.8.2.1 Colour*

The colour of kombucha was primarily due to polyphenols extracted from tea. The characteristic colour of black tea is caused by the enzymatic oxidation of polyphenols by polyphenol oxidase (PPO), associated with the fermentation of "fresh" tea leaves. Fermentation or PPO oxidation polymerizes the original polyphenols found in tea, mainly catechins (epicatechin, epigallocatechin and gallic acid esters), into different classes of polymers [46]. Theaflavins and thearubigins are two polymers that contribute to the black tea colour. Theaflavin, a red-orange dimer, is primarily responsible for the black tea colour, while the higher polymerization level thearubigin is the main pigment. According to Muniz *et al.,* [50], product colour can play a crucial role in the perception of product taste, as people tend to associate specific colours with certain tastes.

Based on the statistical analysis in Table 13, there were no significant differences ($p$ > 0.05) in colour preference scores. All samples K, R1, R2 and R3 received scores around 7, indicating moderate liking. Several panellists noted that the colours of each sample were similar and difficult to distinguish. Sample R2 which was pasteurized, had a moderate liking score. According to Jayabalan *et al.,* [1], kombucha heated above 70°C become darker and clearer. Regular kombucha typically has a cloudy appearance. The sensory test results and comments from the panellists suggest that the colour change in the pasteurized sample R2 was not pronounced.

#### *3.8.2.2 Aroma*

No studies have focused on volatile organic compounds (VOCs) in kombucha. Kombucha is often described as "cider-like." The typical tea aroma profile does not significantly influence the distinctive aroma profile of kombucha. Instead, the dominant aroma profile is controlled by acetic acid and VOCs produced by yeast [39]. According to Table 13, there were no significant differences ($p$ > 0.05) in aroma preference scores. On average, all samples K, R1, R2 and R3 received a score of 5, indicating a neutral aroma. However, sample R2 received the highest score, around 5.88 ± 2.05, indicating a slightly preferred aroma. Some panellists commented that the aroma of kombucha was less pleasant. The pasteurized sample R2 may be more preferred because the fermentation process was halted, resulting in a cider-like aroma.

*3.8.2.3 Sweetness*

The sweetness of kombucha comes from sugars in the raw material and their metabolites, including sucrose, glucose and fructose [19]. According to Table 13, there was a significant difference ($p < 0.05$) in sweetness preference scores between K and R1, R2 and R3. K scored higher in sweetness preference with a score of 6.86, indicating a slight liking, compared to R1 and R3, which received a score of 5, indicating neutral liking. Meanwhile, R2 had the lowest preference score of 4.80 in the category of slight dislike. This significant difference in K scores was due to the higher sucrose content used to brew the tea before fermentation at 9 % compared to 5 % for R1, R2 and R3. Based on panellist comments, the sweetness of sample K was preferred.

The lower preference score for R2 may be due to the findings of Cohen *et al.,* [51] that fermentation temperature and sucrose concentration affect the sensory attributes of kombucha. Although both fermentation temperature and sucrose concentration significantly correlate with overall acceptance and sensory profile reported for kombucha, temperature has a greater influence than sucrose concentration. Kombucha tapped at lower temperatures receives higher acceptance scores. Higher fermentation temperatures accelerate fermentation rates, resulting in higher acidity, causing the sweetness of kombucha to diminish, which cannot be compensated for by higher sucrose concentrations.

*3.8.2.4 Sourness*

The sour taste in kombucha primarily comes from organic acids, including acetic acid, gluconic acid and citric acid [19]. According to Bishop *et al.,* [38,39], acetic acid is one of the major organic acids found in kombucha, produced by AAB often described as tart or sour. Based on Table 13, there was a significant difference ($p < 0.05$) in sourness preference scores for K. K had the highest score of 6, indicating a slight liking for sour taste. This was followed by R3, R1 and R2. R2 had the lowest score of 5.06 ± 1.90 in the 9-point scale, indicating a neutral liking. This may be due to higher fermentation temperatures, which accelerate fermentation rates, resulting in higher acidity.

*3.8.2.5 Bitterness*

Caffeine and polyphenols in tea are major contributors to bitterness in kombucha, often masked by increasing sweetness levels [39]. According to Table 13, there were no significant differences in bitterness preference scores ($p > 0.05$). All samples K, R1, R2 and R3 received similar scores around 5, indicating neutral preference. Sample K had the highest bitterness preference score, 5.94 ± 1.99, possibly because of its high sugar content masking the tea bitterness. This was followed by R3, R1 and R2, which had lower sugar content, resulting in pronounced tea bitterness due to the sugar added to the tea being completely consumed during the fermentation process [39].

*3.8.2.6 Overall acceptance*

The results of the overall acceptance analysis of the sample preference scores showed significant differences ($p < 0.05$) for K. K had the highest score, 6.92 ± 1.64, indicating a slight preference. Considering all the attributes evaluated overall, K received the highest scores for each stated attribute. This was followed by R1, R3 and R2, indicating that the pasteurized kombucha beverage is less liked by consumers with a score of 5.40 ± 1.70.

**Table 13**
Sensory evaluation analysis of kombucha

| Attributes/ Sample | Colour | Aroma | Sweetness | Sourness | Astringent | Overall acceptance |
|---|---|---|---|---|---|---|
| K | 7.32 ± 1.24[a] | 5.52 ± 2.12[a] | 6.86 ± 1.71[b] | 6.42 ± 1.63[b] | 5.94 ± 1.99[a] | 6.92 ± 1.64[b] |
| R1 | 7.06 ± 1.41[a] | 5.56 ± 2.12[a] | 5.26 ± 1.84[a] | 5.62 ± 1.97[ab] | 5.66 ± 1.73[a] | 6.04 ± 1.50[a] |
| R2 | 7.14 ± 1.43[a] | 5.88 ± 2.05[a] | 4.80 ± 2.07[a] | 5.06 ± 1.90[a] | 5.18 ± 1.73[a] | 5.40 ± 1.70[a] |
| R3 | 7.00 ± 1.53[a] | 5.16 ± 2.14[a] | 5.28 ± 2.09[a] | 5.58 ± 1.66[ab] | 5.58 ± 1.58[a] | 5.62 ± 1.56[a] |

***Note:*** K = control, R1 = cold shock, R2 = pasteurization, and R3 = yeast removal. Values are expressed as mean score ± standard deviation. Attributes were assessed on a scale of 9, where: Score 1 = Extremely dislike; Score 2 = Very dislike; Score 3 = Moderately dislike; Score 4 = Slightly dislike; Score 5 = Neutral; Score 6 = Slightly like; Score 7 = Moderately like; Score 8 = Very like; and Score 9 = Extremely like. Values with different superscripts within a row are significantly different ($p < 0.05$)

### *3.9 Kombucha Instant Powder Analysis*

#### *3.9.1 Yield percentage*

According to Sundararajan *et al.*, [52], spray drying process was used to remove liquid from suspensions, emulsions or solutions (feed) to produce dry solids by spraying the feed into a hot drying medium. During spray drying, some particles, especially those with high liquid content, can adhere to the inner walls of the spray dryer, causing wall fouling. Powder sticking to the walls results in agglomeration of the powder and reduces the yield of the spray-dried product. Yield is defined as the ratio of wet spray product collected from the cyclone to the total solids in the feed solution entering the spray dryer. Reduced yield leads to product losses and requires cleaning time for the spray dryer. Wall fouling also affects product quality if the powder on the walls is sensitive to prolonged exposure to temperature during subsequent production.

Based on Table 14, there was a significant difference ($p < 0.05$) in yield percentage values among the samples. The yield percentage for K, 17.13 %, was the lowest. The highest yield percentage was for R3, 38.18 ± 1.94, followed by R1 and R2. This significant difference may be due to the high viscosity of sugar in sample K, causing more sticking to the walls of the spray dryer machine. According to Bhandari *et al.*, [53], the sticking behaviour depends on the sugar content and product temperature. The physical properties of sugars in food affect the drying process differently.

#### *3.9.2 Water Activity*

Water activity is the measurement of water availability for biological reactions and is mathematically related to the ratio of vapor pressure of water in food to the vapor pressure of pure water. It is considered the most critical factor for microbial growth. Fresh foods, which have high moisture content, often have water activity close to 0.99 and are more prone to microbial growth. To prevent microbial growth, water activity needs to be kept below approximately 0.60 - 0.65.

Safe storage for high moisture content foods becomes more challenging due to high ambient temperatures and often inadequate storage and refrigeration facilities. Therefore, it is common to dry food products to reduce food spoilage. Food drying reduces the available moisture to support microbial growth, thereby enhancing the shelf life of the product, which is an ideal solution when appropriate storage is not available [40]. Based on Table 14, water activity analysis shows no significant difference ($p > 0.05$). All samples have a water activity value of < 0.60. This indicates that the water activity of samples K, R1, R2 and R3 were low and meets safe standards to prevent microbial growth.

### *3.9.3 Colour*

The L*, a*, b* values refer to the CIELAB colour space that describes colour based on three axes: L* (brightness), a* (red-green axis, +a* towards red, -a* towards green), and b* (yellow-blue axis, +b* towards yellow, -b* towards blue). These values were used to measure and convey colour information quantitatively [19]. Based on Table 14, the L*, a*, b* colour analysis showed no significant difference ($p > 0.05$). The high positive L* values for all samples indicate brighter brightness for samples K, R1, R2 and R3. The low positive a* values for all samples indicate slight redness in samples K, R1, R2 and R3. The positive b* values for all samples indicate the presence of yellow colour in samples K, R1, R2 and R3. In overall, kombucha has a bright yellow colour.

### *3.9.4 Solubility*

One of the important properties of powder is solubility. Studying the behaviour and response of products in the aqueous phase is valuable, functional and productive. High solubility properties for food powders are important [27]. Based on Table 14, the percentage solubility analysis shows no significant difference ($p > 0.05$). All samples have low solubility percentages. This indicates low solubility. If the percentage of solubility is high, it indicates that most of the kombucha powder dissolves in the solvent, suggesting high solubility. Conversely, low solubility percentage indicates that only a small portion of the powder dissolves, indicating low solubility.

**Table 14**
Kombucha powder analysis

| Sampel | Yield (%) | Water activity ($a_w$) | Colour | | | Solubility (%) |
|---|---|---|---|---|---|---|
| | | | L* | a* | b* | |
| K | 17.13 ± 6.73$^{a}$ | 0.38 ± 0.04$^{a}$ | 86.37 ± 0.54$^{a}$ | 3.26 ± 0.18$^{a}$ | 18.41 ± 0.68$^{a}$ | 0.18 ± 0.03$^{a}$ |
| R1 | 31.90 ± 4.54$^{ab}$ | 0.35 ± 0.04$^{a}$ | 85.59 ± 1.58$^{a}$ | 3.59 ± 0.38$^{a}$ | 19.39 ± 2.04$^{a}$ | 0.12 ± 0.02$^{a}$ |
| R2 | 30.30 ± 7.54$^{b}$ | 0.31 ± 0.02$^{a}$ | 85.72 ± 0.43$^{a}$ | 3.58 ± 0.37$^{a}$ | 18.43 ± 1.31$^{a}$ | 0.15 ± 0.02$^{a}$ |
| R3 | 38.18 ± 1.94$^{b}$ | 0.35 ± 0.04$^{a}$ | 87.46 ± 0.55$^{a}$ | 2.88 ± 0.12$^{a}$ | 16.81 ± 1.34$^{a}$ | 0.15 ± 0.03$^{a}$ |

***Note:*** K = control, R1 = cold shock, R2 = pasteurization, and R3 = yeast removal. Values are expressed as mean score ± standard deviation. Values with different superscripts within a row are significantly different ($p < 0.05$)

## 4. Conclusion

This study successfully explored methods to halt alcohol production during kombucha fermentation and developed instant kombucha tea using spray drying techniques. Physicochemical and proximate characteristics were determined, followed by sensory evaluation through hedonic testing with 50 panellists. Significant outcomes were achieved in the production of instant kombucha powder, with pasteurization reducing alcohol content to 0.13 % w/w in sample R2. Sample R2 exhibited 4.70 ± 0.10 total dissolved solids, pH 3.10 ± 0.17 and titratable acidity of 0.2177 ± 0.0216. Proximate analysis revealed high moisture content (95.0568 ± 0.5289 %) and carbohydrates (4.7142 ± 0.5208 %), low protein (-0.0179 ± 0.0108 %) and moderate ash (0.2469 ± 0.0189 %).

Hedonic testing indicated that sample R2 received moderate ratings for colour (7.14 ± 1.43) and overall acceptance (5.40 ± 1.70), with a preference noted for its sour taste (5.06 ± 1.90) over sweetness (4.80 ± 2.07). Pasteurized kombucha, particularly sample K, showed high consumer acceptance across all attributes. Spray drying produced instant kombucha powder with a yield percentage of 30.30 ± 7.54 %, water activity of 0.31 ± 0.02 and notable colour characteristics (L* 85.72 ± 0.43, a* 3.58 ± 0.37, b* 18.43 ± 1.31). Sample R2's low water activity and bright yellow colour highlight its safety from microbial growth. Pasteurization and spray drying effectively enhance

kombucha's shelf life, making it suitable for commercial energy drinks due to its high carbohydrate content.

In overall, pasteurized kombucha particularly sample R2, is well-suited for commercial purposes due to its low alcohol content, stability against microbial contamination and extended shelf life through spray drying. These methods prevent secondary fermentation post-production, ensuring product safety and consumer acceptance. Also, instant kombucha powder particularly sample R2, exhibits promising suitability for incorporation into kombucha ice cream as a special flavoured ice cream not yet available in the market. The spray drying process effectively preserves its physicochemical properties, including low alcohol content (0.13 % w/w), high carbohydrate content and stability against microbial growth with a low water activity. The bright yellow colour and positive sensory attributes observed through hedonic testing indicate consumer acceptance, particularly for its sour taste profile. Incorporating instant kombucha powder into kombucha ice cream could offer a unique, health-conscious dessert option enriched with probiotics and distinctive flavour characteristics, appealing to consumers seeking innovative and nutritious frozen treats.

## 5. Recommendations for Future Research

Future research in kombucha production should prioritize enhancing product quality and safety, with a specific focus on developing instant kombucha drink and kombucha ice cream using advanced spray drying techniques. The key area of exploration includes:

a) Implementing cooling treatments supplemented with anti-mold preservatives e.g. 0.1 % sodium benzoate and 0.1 % potassium sorbate in kombucha with pH ≤ 4.2 stored below 5°C, can effectively inhibit mold and yeast growth, though it may not entirely halt alcohol production.
b) Advanced filtration technologies such as Plate and Frame Filtration should be investigated for their ability to remove yeast and SCOBY granules, improving beverage clarity and reducing contamination risks compared to traditional methods.
c) Detailed microbial analyses are essential to identify yeast strains and characterize mold contaminants, ensuring the safety and suitability of kombucha for consumption.
d) Optimizing fermentation durations to minimize alcohol production while maintaining desired physicochemical, proximate and sensory characteristics is critical. Comparative studies across varied fermentation periods will provide valuable insights for refining production practices and meeting consumer preferences.
e) For the development of low alcohol kombucha, researching the impact of different sugar levels and longer fermentation times on maintaining low alcohol content and product quality is crucial. Managing fermentation temperatures effectively regulates yeast activity and minimizes alcohol production, meeting the demand for low-alcohol beverages.
f) Regular quality control checks throughout fermentation and post-production are necessary to monitor alcohol content and comply with regulatory standards.
g) Future research should also look into innovative spray drying techniques and ingredients to enhance the sensory qualities and nutritional benefits of instant kombucha drink and kombucha ice cream.
h) Long-term studies on storage conditions' effects on microbiological stability and shelf life will contribute to developing high-quality, consumer-accepted products that meet regulatory standards and offer significant health benefits.

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