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EFFECT OF CALCIUM CHLORIDE CONCENTRATION ON IMPROVING THE QUALITY OF RESTRUCTURIZED STRAWBERRY FRUIT (*Fragaria x ananassa*)

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ABSTRACT

Strawberry is a very popular fruit due to their bright red color and aroma. sweet and slightly sour taste and nutritional value, especially vitamin C. Strawberries also contain anthocyanins, a reddish-purple pigment with high antioxidant activity. Nevertheless, they have a relatively short shelf life and are easily damaged because of their soft texture. They are usually processed into jam and other products that use high temperatures, which can damage nutritional compounds, including anthocyanins. Restructuring is an alternative method that can be used to extend the shelf life of strawberries without heat, thereby avoiding damage to the nutritional value and antioxidant activity. This study aimed to determine the effect of various Alginate and $CaCl_2$ concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) on the quality of restructurized strawberries. The anthocyanin content was determined using the pH differential method; the texture was determined using a simple test to distinguish differences between treatments; the organoleptic test used hedonic methods with 30 panelists; and the shelf life tests were used to keep the real product until the quality decreased. Preliminary experiments showed that 2% alginate was optimal. The second step showed that 0.4% $CaCl_2$ was optimal.

Keywords: alginate, anthocyanin, $CaCl_2$, restructurized, strawberry

INTRODUCTION

Strawberries are highly popular in Indonesia, despite not being native to the region. They are widely cultivated across Indonesia and consumed by people of all ages, both fresh and processed into various products such as jam, syrup, dodol (traditional confectionery), preserved fruit, juice, and ice cream (Zaimah et al., 2013; Susianti, 2015)

Processing strawberries has been widely practiced for many years to prolong the shelf life of food products, including jams, preserved fruit, syrups, and 'dodol'(sweet treat). All of these processing methods require heating, which can destroy vitamin C and anthocyanins that are susceptible to heat, thus reducing their nutritional value. In addition to heating, processed jams, syrups, and dodol require the addition of significant amounts of sugar, making them unsuitable for diabetics. Another method to extend the shelf life of strawberries is through restructuring.

Restructuring is a physical and chemical process that reshapes the structure of food using a binder into a 'jelly like product' that meets consumer desires in terms of shape, size, texture, appearance, and taste. One example of a gel system as a chemically induced (non-thermal) binder is a combination of alginate with calcium ions. Fruit restructuring can be performed using sodium alginate with calcium lactate or calcium chloride (Raharjo and Utama, 2002). The restructurized method has been applied to jambolan fruit (Herawati et al., 2016) and strawberry fruit (Lestario, 2022). However, these studies achieved limited shelf life extension (3-4 days in a refrigerator), indicating the need for optimization of alginate and calcium chloride concentrations.

This study aimed to (1) determine the optimal concentrations of sodium alginate and $CaCl_2$ for strawberry restructuring and (2) evaluate the effects of these concentrations on anthocyanin retention,

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texture, and sensory acceptability of the restructurized product.

MATERIAL AND METHODS

Material specifications

The materials used in this research were sodium alginate, calcium chloride ($CaCl_2$), HCl, sodium acetate, KCl, ethanol (Merck, Germany), and granulated sugar (Gulaku). Strawberries (mature, red) were purchased fresh from a traditional market in the Getasan district on the Kopeng-Salatiga road, brought to the laboratory, cleaned from dirt (dust, sand, and leaves), washed under tap water, decanted for approximately 5 min, and then processed for restructurized fruit.

The tools include: analytical balance (OHAUS Pioneer PA214); mixer (Cosmos - 1279); UV-VIS spectrophotometer (Genesys, USA); cuvette; oven; desiccator; cutting board and knives.

Experimental Design and Analysis

The experiment was arranged in a Completely Randomized Design with six levels of calcium chloride concentration (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5% w/v) as the independent variable, while sodium alginate concentration was fixed at 2% w/v based on preliminary optimization. Each treatment was replicated thrice, resulting in 18 (6 × 3) experimental units. Data on anthocyanin content, texture properties, and sensory attributes were subjected to analysis of variance (ANOVA) using SPSS version 25.0, IBM Corp., USA, followed by the Least Significant Difference (LSD) test at 95% confidence level ($\alpha = 0.05$).

Research Procedure

Preparation of Restructurized Strawberry

Restructurized strawberry was prepared following the method of Lestario et al. (2020) with modifications. Fresh strawberries were washed, hulved, and homogenized into pulp. A total of 100 g strawberry fruit pulp was mixed with 2% (w/w) sodium alginate and homogenized using a blender for 5 min. Granulated sugar (7 g) was added and mixed for 1 min. Subsequently, calcium chloride solution at varying concentrations (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5% w/v) was added and homogenized for 2 min. The mixture was transferred to containers and stored at 4°C for 2 h to allow complete gel formation.

Determination of Texture

In the absence of a texture analyzer, the texture was evaluated using a penetration test with a standardized toothpick. A wooden toothpick was pressed vertically into the product at three different locations using consistent manual pressure. Resistance to penetration was assessed and rated on a 5-point scale as follows: 1 = very soft and watery, 2 = soft, 3 = moderately firm, 4 = firm, and 5 = very firm. Three replicate measurements were performed for each sample. Although semi-quantitative, this method provided comparative data among treatments. Future studies should use instrumental texture analysis for more objective measurements.

Anthocyanin Content Determination

The total anthocyanin content was measured using the pH differential method (Cheng and Breen, 1991). Samples (2 g) were macerated with 60 mL methanol-HCl 1% in a refrigerator (10 °C) overnight, filtered, and the waste was re-extracted twice with the same solution (2 × 20 mL). The filtrate was collected in a 100 mL flask, and the same solution was added to the calibration mark. Several milliliters of the anthocyanin extract were diluted with pH 1.0 buffer (0.025 M KCl) and pH 4.5 buffer (0.4 M sodium acetate) for comparison. The absorbance was measured at 510 and 700 nm using a UV-Vis spectrophotometer. Anthocyanin concentration was calculated using the Lambert-Beer equation:

$$A = \varepsilon\, b\, c;$$

where:

A = $[(A510\text{-}A700)\,pH1 - (A510 - A700)\,pH4{,}5]$
ε = molar extinction coefficient of cyanidin 3-glucoside (29,600 L mol^{-1} cm^{-1})
b = path length (1 cm)
c = anthocyanin concentration (mol/L).

The results were expressed as mg cyanidin-3-glucoside equivalents per 100 g of sample.

Determination of Water Content (Oven Method)

The water or moisture content of the 'restructurized fruit' was determined by drying an empty cup in an oven at 105 °C overnight and then cooling it in a desiccator. A 2 g sample of 'Restructurized Fruit' was placed in the cup and dried in an oven at 105 °C for 4– 6 h, cooled in a desiccator, and weighed again until constant. The water content was calculated using the following formula:

$$\text{Water content (\% wb)} = \frac{\text{Water weight}}{\text{Initial weight}} \text{ x } 100\%$$

Organoleptic Test

Organoleptic tests, including color, aroma, taste, texture, and overall, were conducted according to Soekarto (1985). This was done using a hedonic test with 30 panelists. The scale used was as follows: 1 = dislike very much, 2 = dislike, 3 = somewhat like, 4 = like, and 5 = like very much.

Shelf Life Test

The shelf life of the restructurized strawberries was determined by storing the product in a refrigerator and observing its quality (color, aroma, and flavor) (Yusuf, 2023). The samples were checked for mold every 3 days until the quality decreased (different color, aroma, and flavor). Once the sensory attributes became abnormal or any mold was observed, the observation stopped, and the shelf life was determined. Future research will observe the samples every day to obtain a more accurate shelf life.

RESULTS AND DISCUSSION

Texture

The texture o f the restructurized fruit with the addition of 2% sodium alginate and various concentrations of calcium chloride (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5) is shown in Table 1.

Table 1. Effect of calcium chloride concentration on texture, anhocyanin content and water content of restructurized strawberry fruit products

$CaCl_2$ (%)	Texture	Anthocyanin Content (mg/100 g)	Water Content (%)
0.0	1.00 ± 0.00^{a}	27.52 ± 0.98^{a}	80.12 ± 0.01^{e}
0.1	2.17 ± 0.29^{bc}	26.48 ± 0.99^{a}	71.30 ± 0.52^{c}
0.2	2.67 ± 0.29^{bc}	24.23 ± 6.30^{a}	76.28 ± 0.04^{d}
0.3	3.00 ± 1.00^{c}	24.18 ± 1.90^{a}	69.20 ± 0.23^{b}
0.4	3.17 ± 0.76^{c}	22.81 ± 4.69^{a}	64.96 ± 0.11^{a}
0.5	1.92 ± 0.38^{ab}	22.11 ± 1.38^{a}	76.76 ± 0.71^{d}

Notes: Data (mean± standard deviation) were obtained from three replicates. Data were analyzed using ANOVA. Data within the same column followed by different letters indicate a significant difference (LSD test, $p<0.05$).

The Results showed that the higher the concentration of $CaCl_2$, the firmner (harder) the texture, it can be said that increasing concentration of $CaCl_2$ can improve the texture or make the restructurized fruit harder, it was good because it can increase the shelf life of the restructurized product.

Alginate and $CaCl_2$ formed a crosslinking bond, where the Ca^{2+} ion binds two adjacent alginate molecules to form an 'egg box', so that the sodium alginate, which was originally liquid (soluble in water), formed a solid gel structure. Based on the bond type, more Sodium Alginate is needed than $CaCl_2$ because Ca^{2+} is only needed as a connector between two alginates. Thus, the more $CaCl_2$ is added, the more cross-links (egg boxes) are formed in the gel, which causes the texture of the product to become harder or stronger until it reaches its optimum (Lestario et al., 2016; Maharani et al., 2022). If $CaCl_2$ is still added, it will destroy the gel; the newly added $CaCl_2$ will pull the $CaCl_2$ in the eggbox, destroying the crosslinking bond. Therefore, the texture of the samples treated with 0.5% CaCl2 decreased sharply because of the destruction of the crosslinking and egg box structures.

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Anthocyanin Content

The anthocyanin content of the restructurized strawberry fruit is presented in Table 1. The results showed that the higher the concentration of $CaCl_2$, the lower was the anthocyanin content. However, the differences between the concentrations were not significant. Increasing the concentration of $CaCl_2$ tended to decrease the anthocyanin content of restructurized strawberry fruit slightly, but not significantly.

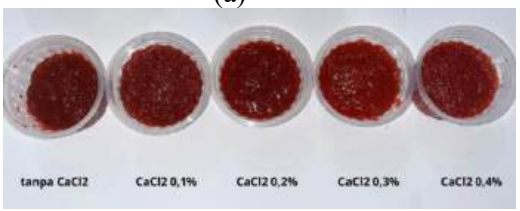
Furthermore, the visual appearance did not show a decrease in the purple/red color, which reflected a decrease in anthocyanin. with increasing $CaCl_2$. It was estimated that the anthocyanin was still in the product or not degraded with the increasing CaCl2 concentration. However, it was not extracted in anthocyanin determination because anthocyanin compounds can be bound to calcium alginate hydrogels (eggbox alginate-$CaCl_2$) through ionic interactions, where calcium ions (Ca^{2+}) help form a strong alginate gel structure and also interact with anthocyanins, causing some anthocyanins to be trapped or bound within the gel matrix, resulting in reduced levels when measured using standard extraction methods of anthocyanins. However, the non-extracted anthocyanin was still in the product, and when the restructurized fruit was consumed, the anthocyanin was still in the product and can benefit the human body as an antioxidant (Herawati et al., 2016).

Water Content

The higher concentration of $CaCl_2$, affected insignificantly on water content of the product Table 1.). It decreased in 0.1% $CaCl_2$ but increased in 0.2% $CaCl_2$ and then decreased again in 0.3% $CaCl_2$. In other words, the water content significantly increased with increasing $CaCl_2$ content, from 76.76 to 80.12%.

According to the Indonesian National Standard (SNI), the water content of 'jelly like' food should be around 20%. The water content in this study was too high; therefore, it needs to be developed again to reach the Indonesian National Standard. However, the appearance of the product was satisfactory to the panelists in the organoleptic test. They did not appear too moist and looked better and former compared (than) those in previous studies (Figure 1a and 1b).

(a)



(b)

Figure 1. Appearance of restructurized strawberry fruit. Previous researh (a), this research (b)

Organoleptic Test

The results of the organoleptic tests of the restructurized strawberry fruit are presented in Table 2. The results of the organoleptic test showed that the higher the concentration of $CaCl_2$, the color preference of the panelists was not affected; however, it did have an effect on the aroma, texture, taste, and overall. The aroma had tendency to decrease or less prefered by the panelist, the most prefered for aroma was 0.1% $CaCl_2$; The most prefered texture was 0.2-0.4% $CaCl_2$; the most prefered taste was 0.1-0.3% $CaCl_2$; the most prefered for overall was 0.4% $CaCl_2$. From all the results of the organoleptic test, the most important factor was taste; therefore, 0.4% $CaCl_2$ could be the best concentration.

Shelf Life

The results of the shelf life test of restructurized fruit can be seen in Table 3, which showed that the product can be stored in the refrigerator for up to 9 days. From day 0 to day 9, the color was red in all concentrations, there was no mold, the aroma was the same as at the beginning (strawberry aroma), and the flavor/taste was sweet and slightly sour.

However, on day 12th, the color of the products was still red, but some part of the surface of the product with 0.0, 0.1, 0.2, and 0.3% $CaCl_2$ was covered by white mold, whereas the product with 0.4% and 0.5% $CaCl_2$ was not covered by mold. This can be explained by the fact that the products with

0.4 and 0.5% $CaCl_2$ had higher Aw than those with 0.0, 0.1, 0.2, and 0.3% $CaCl_2$. This is in accordance with the theory that the higher the Aw, the more difficult it is for microbes to grow (Winarno, 1987). The aroma became sour at all concentrations, indicating that the products began to decay; the flavor was not tested for safety. In future research, shelf life observations will be performed every day to obtain a higher thorough. In this case, the product may have still been good on days 10 or 11.

Table 2. Effect of calcium chloride concentration on organoleptic characteristics of the restructurized strawberry fruit

$CaCl_2$ (%)	Color	Aroma	Texture	Taste	Overall
0.0	3.60±0.67^{a}	3.70±0.84^{b}	2.53±0.63^{b}	3.70±0.95^{b}	3.50±0.51^{b}
0.1	3.67±0.66^{a}	3.70±0.84^{b}	2.87±0.51^{bc}	3.57±1.01^{b}	3.50±0.51^{b}
0.2	3.73±0.69^{a}	3.60±0.81^{ab}	3.07±0.37^{c}	3.60±0.62^{b}	3.77±0.77^{b}
0.3	3.73±0.64^{a}	3.57±0.86^{ab}	3.03±0.93^{c}	3.60±0.86^{b}	3.57±0.50^{b}
0.4	3.53±0.73^{a}	3.37±0.89^{ab}	3.17±0.59^{c}	3.50±0.78^{b}	4.20±0.76^{c}
0.5	3.37±0.81^{a}	3.13±1.04^{a}	2.03±0.76^{a}	2.50±0.78^{a}	2.63±0.56^{a}

Notes: Data (mean±SD) were interval data from transformed ordinal data of organoleptic hedonic responses. The data were analyzed using ANOVA. The data within the same column followed by different letters are significantly different (LSD test, $p<0.05$).

Table 3. Shelf life test of restructurized fruit

Day	Color	Microbes	Aroma	Flavor
0	Red	-	Fruity (Strawbery)	Sweet
3	Red	-	Fruity (Strawbery)	Sweet
6	Red	-	Fruity (Strawbery)	Sweet
9	Red	-	Fruity (Strawbery)	Sweet
12	Red	0%-0.3%: + 0.4%-0.5%: -	Sour +	Not tested
15	Red, covered by slighty white microbes	0%-0.3%: ++ 0.4%-0.5%: +	very sour ++	Not tested

Strawberries are fruits with a very short shelf life, often classified as a 'very perishable' fruit with characteristics of high water content, soft and thin peel, and high ethylene production before ripening, which results in a high respiration rate, and therefore easy decay (perishable). Therefore, this type of fruit requires good handling to extend its shelf life or to make a product based on this fruit with no heat to avoid the decrease in nutrition, including anthocyanin and vitamin C. This method is called restructurization, and the product is called restructurized fruit, which extends the shelf life with minimal processing. Compared to the original fruit, which usually only lasts 1-2 days in the refrigerator, the restructurized strawberry fruit can extend the shelf life up to 9 days in the refrigerator.

On the 12th day, the appearance became moldy on the surface area, covered by slightly mold; the aroma became sour; and the taste was not tested due to safety. Therefore, it can be concluded that the shelf life of the restructurized product was nine days. However, the product was still good on day 10 or 11, but not observed. Further research will observe the product every 2 days or every day to obtain more accurate data.

CONCLUSIONS

The optimal concentration of $CaCl_2$ that produced the best restructurized strawberries was 0.4% (for 2% sodium alginate). At this concentration, the anthocyanin content was 22.81 ± 4.69 mg/100 g; the texture score was 3.17 ± 0.76 (scale 1-5, hardest among other concentrations); for sensory acceptability, the

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color score was 3.53±0.73; aroma was 3.37±0.89; texture was 3.17±0.59 (most preferable compared to other concentrations); taste was 3.50±0.78; overall was 4.20±0.76 (most preferable compared to other concentrations). In addition, this study yielded better results than previous studies, especially in terms of firmer texture and longer shelf life (from 1 d to 9 days).

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RESTRUCTURING VEGETABLES INTO EDIBLE FOLDING PAPER TO IMPROVE NUTRIENT INTAKE

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ABSTRACT

Vegetable consumption in Indonesia is low, particularly among preschool-aged children, who often do not meet daily intake recommendations. Sensory factors, such as taste, texture, and appearance, strongly influence children's acceptance of vegetables. Previous studies have shown that modifying vegetables and using play-based learning can improve children's interest. This study aimed to develop an edible folding paper made from vegetables, serving as both a nutritious food product and an interactive learning tool. Spinach, purple cabbage, and pumpkin were chosen for their good acceptance, nutrient content, and color appeal. The product was analyzed for macronutrients (proximate analysis) and micronutrients, including vitamin C, antioxidant activity, and iron (Fe) content. The results showed adequate levels of energy, protein, fat, and carbohydrates. Micronutrient analysis indicated 3.31 mg of iron, 53 mg of vitamin C, and 56.21% antioxidant inhibition per 100 g of product. Texture evaluation using origami-style folding confirmed good flexibility and tear resistance, supporting its function as an edible paper. Overall, the product retained substantial nutrients and could be folded into origami shapes without losing its structural integrity. This innovation has the potential to increase vegetable intake in children and improve dietary quality during early childhood.

Keywords: Antioxidants, Children, Edible paper, Nutrition, Vegetables

INTRODUCTION

Indonesia has very low vegetable consumption across various age groups, including that of school-aged children. A common problem is insufficient fiber intake, including vegetable consumption, which does not meet the body's needs (Ningrum et al., 2024). According to data from the Indonesian Health Risk Survey (Kemenkes RI, 2019), 95.55% of the population aged ≥5 years consumes insufficient amounts of vegetables and fruits (Ministry of Health, 2020). Low vegetable intake among children is often caused by a lack of interest in vegetables. This is influenced by several factors, such as taste, appearance, and eating habits that have been formed since early childhood (Maulani and Retnoningsih, 2025). To overcome this, innovations in vegetable processing are needed in forms that are more appealing to children while retaining the important components in the vegetables. One such innovation is the processing of vegetables into edible origami paper, known as edible folding paper sheets.

Origami paper was chosen because origami provides meaningful benefits for early childhood development by improving fine motor skills, hand–eye coordination, and concentration (Anisa et al., 2021; Hosotani, 2025). It also strengthens cognitive abilities through the introduction of shape recognition, symmetry, and spatial awareness (Anisa et al., 2021; Dwipadmini, 2025).

Edible paper sheets are generally formulated using starch and its derivatives (Karnwal et al., 2025; Utami Hatmi et al., 2020, 2020; Wibowo et al., 2019). Therefore, the use of vegetables and fruits as primary materials for edible film sheets is relatively novel. The ingredients used are vegetables with striking colors, similar to origami paper in general, but with good nutrient content, including green spinach, purple cabbage, and yellow pumpkin. However, the components in vegetables are very sensitive to heat processing (Ho and Redan, 2022; Lee et al.,

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2017); therefore, materials that can reduce the effects of heat are needed, such as fiber. The sugar structure in fiber undergoes dehydration during the heating process (Werner et al., 2014), which then undergoes a hardening structural change (Sikora et al., 2022), protecting from heat penetration (Fliri et al., 2024), but on the other hand, it still provides flexible characteristics and does not easily break the product (Wulandari and Saidi, 2019).

The purpose of this study was to restructure vegetables into foldable sheets, Edible Folding Paper, and apply them to origami shapes while maintaining or minimizing the loss of nutritional components in vegetables. It is hoped that edible folding paper products can help increase vegetable intake in children through play and learning.

MATERIALS AND METHODE

Material

Each vegetable exhibits a distinct dominant nutrient or functional compound profile. Green spinach is particularly rich in iron (Fe), pumpkins contain elevated Vitamin C levels, and purple cabbage is known for its high anthocyanin content, which is a potent antioxidant. The vegetables used in this study were fresh and obtained from local markets in Salatiga City. For the binding agent (agar), a commercially available, unflavored product variant was used.

Experiment Design and Analysis

The research design used an experimental research method. The process stages and binder concentrations used in the formulation were the same. Variations were made in the types of vegetables used. Product analysis includes macronutrient analysis (proximate analysis) and micronutrient analysis, which varies depending on the type of vegetable used: Fe analysis for spinach products, antioxidant analysis for purple cabbage products, and vitamin C analysis for pumpkin products.

All analyses were performed in triplicate, and the data are reported as the mean ± standard deviation. The analytical outcomes were benchmarked against the macro- and micronutrient compositions of spinach, pumpkin, and purple cabbage using the Indonesian Food Composition Table (TKPI) as the reference standard.

Research Procedure

The research stages included material preparation, vegetable puree drying, and product analysis.

Material Preparation (Puree Process)

Vegetables (500 g) were sorted, washed thoroughly, and blanched in hot water before immersion in cold water for approximately 10-15 min. The mixture was then pureed using a blender, and 15 g of a fiber-based binding agent (agar) was added. The binding agent (agar) was evenly mixed with the vegetable puree.

Drying

The vegetable puree was spread on a baking sheet to a thickness of approximately 1 millimeter (1 mm). The samples were dried at a temperature of ±75 °C or at low heat if using a microwave dryer for approximately 75 min. The target product moisture content is below 14%, with the indicator being that the product surface is dry and not sticky.

Analytical Procedure

Macronutrient Analysis (Proximate Analysis)

Moisture Content (AOAC, 2023)

The porcelain dish was cleaned and dried in an oven for 15 min at 105°C, then cooled in a desiccator for 30 min and weighed. Weigh 5 g of the sample and place it in a porcelain dish of known weight. The samples were then dried in an oven at 102-105 °C for 6 h.

Ash Analysis (BSN, 2009)

The porcelain ash dish was cleaned and dried in an oven at 105 °C for 30 min, cooled in a desiccator for 30 min, and weighed. Five grams of the sample was placed in a porcelain ash dish. The dish was placed in an oven at 105 °C for 30 min. Subsequently, it was incinerated in a furnace at 600 °C for 7 h until a gray color was formed. The sample was then cooled in a desiccator for 30 min and weighed. The treatment was repeated until a constant weight was achieved.

Carbohydrate Analysis (FAO, 2003)

For the "carbohydrates by difference" method, 100% was subtracted from the total content of other nutrients.

% Carbohydrate = 100% - (Moisture content + Ash+ Protein + fat)

Fat Analysis (Stefanie et al., 2023)

Fat content analysis was performed using the direct extraction method with a Soxhlet apparatus. The procedure began with drying the extraction flask in an oven, cooling it in a desiccator, and weighing it until its weight was constant. A total of 100 g of the sample was ground and wrapped in a filter paper to form a thimble. The sample was placed in a Soxhlet apparatus with hexane solvent added until 1.5 cycles (150 min) of extraction occurred, which took approximately 6 h until the solvent returned through the siphon into a clear fat flask. The extraction results from the fat flask were separated between hexane and extracted fat using a rotary evaporator at a temperature of 69 °C and a speed of 50 rpm. The fat separated by hexane was then heated in an oven at 105 °C for 1 h. The fat flask was cooled in a desiccator for 15 min and weighed to obtain the weight of the extracted fat. The content can be calculated using the following equation:

$$\% \text{ Fat} = \frac{W_3 - W_2}{W_1} \times 100\%$$

Where are:
W_1 : Sample mass (g)
W_2 : weight of empty Fat flask (g)
W_3 : weight of empty Fat flask + Extracted fat (g)

Protein Analysis (Syahfitri et al., 2025)

Protein content analysis was performed using the Semimikro Kjeldahl method. The sample was crushed and weighed to 1 g and then placed in a Kjeldahl flask. To facilitate the destruction process, 2 g of the mixed catalyst and 25 mL of concentrated H_2SO_4 were added and stirred until homogeneous. The solution was heated to boiling until the color changed to clear green, and then cooled. After cooling, the destruction solution was diluted with 100 ml of distilled water. A total of 5 ml of this diluted solution was pipetted into a distillation flask. Subsequently, 30% NaOH was added until the solution became alkaline, followed by the addition of several boiling stones. Distill the solution for 5-10 minutes. The distillate was collected in an Erlenmeyer flask containing 10 mL of 2% boric acid solution and several drops of a mixed indicator (methylene red + bromothymol blue). Subsequently, the distillate was titrated with 0.02 N hydrochloric acid standard solution, and the titration was terminated when the color changed from blue to orange. The same procedure was repeated to prepare a blank sample as a control. The concentration was calculated using the following formula:

$$\% \text{ N} = \frac{(v_1 - v_2) \times \text{N HCl} \times 0.014 \times \text{fk} \times \text{fp} \times 100}{\text{sample}}$$

Where are :
v_1 : Volume of HCl sample
v_2 : Volume of HCl blanko
N HCl : Normality of HCl Solution
0.014 : Conversion factor of nitrogen to protein
fk : Protein conversion factor
fp : Dilution factor

Fiber Analysis (Hardiyanti and Nisah, 2021)

The determination of crude fiber uses the gravimetric method, which is an analytical technique used to measure the amount of fiber in the sample. The sample was ground and weighed at 2-4 g and weighed on a constant filter paper. The sample was then dissolved in 15 mL of 96% ethanol and stirred for 30 s. The sample was allowed to rest for 15 min and then filtered using a Buchner funnel, and the remaining sediment was rinsed with 45 mL of 96% ethanol. Subsequently, the filter paper was dried in an oven at 105 °C until dry. The sample was then placed in a desiccator for 15 min, and the filter paper with the residue was weighed again. The residue was dissolved in 50 mL of 1.25% H_2SO_4. The sample solution was heated in a water bath at 60 °C for 30 min, and 50 mL of 3.35% NaOH was added, followed by heating again in a water bath. The sample was prepared for filtering using a stabilized filter paper. The residue on each filter paper was washed with 25 mL of hot 25% H_2SO_4, 25 mL of hot distilled water, and 25 mL of 96% ethanol. The residue was placed in a dish and dried again in an oven at 105 °C. The mixture was cooled in a desiccator for 15 min. The constant weight was calculated. Crude fiber analysis was performed using the following formula:

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$$\text{Crude Fiber}(\%)=\frac{\text{(W. filter paper+sample)-W. filter paper}}{\text{W. Sample}}\times 100\%$$

Where are :
W Filter paper + sample: weight of dried filter paper + sample (g)
W Filter paper : Weight of filter paper (g)
W sample : Weight of sample (g)

Micronutrient Analysis

Vitamin C (Fitriana and Fitri, 2020)

Sample preparation

To 10.00 g of the sample, 100 mL of 3% (w/v) metaphosphoric acid was added. The mixture was homogenized until uniform, and then centrifuged to obtain a clear extract.

Titration process

The sample (10 mL) was added to a 100 ml measuring flask, distilled water was added, and the mixture was homogenized. Then, 10 mL of the sample was taken, 2 mL of amylum solution was added, and the mixture was titrated with 0.01 N I_2 solution until the color changed to blue violet.

Antioxidant activity (%inhibition)

The sample extract (1 mL) was added to 1 ml of DPPH solution and incubated in the dark for approximately 30 min. Absorbance was measured at 517 nm. Compare with the blank. The inhibition percentage was calculated using the following formula:

$$\%\ \text{Inhibition}=\frac{\text{Abs. Control-Abs. Sample}}{\text{Abs. Control}}\ \text{x } 100\%$$

Fe Content Analysis (Putri et al., 2020)

Then, 10 mL of concentrated HNO_3 was added to 1 g of the sample, and the mixture was heated until fully dissolved. The mixture was allowed to cool, and distilled water was added to a volume of 100 ml. The absorbance of the standard solution was measured from the lowest to the highest concentration at a wavelength of 248.3 nm. Measurements are performed using the test sample. If the test sample reading is outside the calibration range, the test sample should be diluted and measured again.

Fold Test (Widiastuti et al., 2024)

The test was performed by folding the product repeatedly at the same spot until it broke at the fold. The number of folds that can be made before it breaks is counted. If the product can be folded more than 100 times before breaking, it has excellent fold resistance.

RESULT AND DISCUSSION

Macronutrient Analysis (Proximate)

The edible folding paper vegetable sheets in Figure 1 visually show that the original colors of fresh vegetables are still well expressed, including the green of spinach, purple of cabbage, and yellow of pumpkin. This is an initial indication that the nutrient content in vegetables is still largely protected. The addition of agar as a binding agent not only provides flexibility to the texture of the Edible Paper product but also protects the color and bioactive components of the vegetables during the drying process (Santoso, 2024; Ziedan et al., 2018). The average weight per product for each variant was approximately 12 g per sheet, with sheet dimensions of 15 cm × 20 cm.

To ensure the content of Edible Paper products, micro-and macronutrient content analyses were conducted on the products. The results of the macronutrient analysis in the form of proximate tests for each Edible Folding Paper product are presented in Table 1.

Figure 1. The edible folding paper of vegetables

Table 1. Proximate Analysis of Edible Folding Paper

Parameter	Spinach	Yellow Pumpkins	Purple Cabbage
Ash	9.91 ±0.33	9.81±0.33	8.70±0.21
Moisture content	13.09±0.41	10.64 ±0.55	7.35±0.44
N-Total of Protein	6.85 ±0.18	7.46 ± 0.18	3.68±0.17
Fat	0.32±0.02	0.34±0.01	1.69±0.02
Carbohydrate	34.75 ±0.44	39.78±1.00	43.26±0.96
Fiber	35.07 ±0.67	31.97±0.38	36.32±0.51

Note: Data are presented as percentages per 100 g of sample.

The analytical results for each variable across all sample types demonstrated excellent standard deviation values, which did not exceed 10% of the mean. Accordingly, the data distribution was considered stable and reliable.

The moisture content of the Edible Paper products listed in Table 1, based on proximate analysis, ranged from 7 to 13%. This value is in line with the initial target of below 14% (Akther et al., 2023). The ash content of the product was generally higher than that of similar dried vegetable products (Ambo et al., 2023; Ashfaq, 2018; Surya et al., 2021). This may be influenced by the high proportion of the binding agent (agar) added to the formulation, which was 15%. At this range, it can affect the high ash content produced (FAO, 2003). The N-total protein content in the product ranges from 3-6%. This value was lower than that of dried spinach (Waseem et al., 2021), dried pumpkin (Bemfeito et al., 2020), and dried purple cabbage (Zhang et al., 2021). This is likely influenced by the amount of binding agent (agar) added, which is a hydrocolloid that increases the total non-protein solids in the product (Waseem et al., 2021). The fiber content of Edible Paper products range from 31-36%. The high fiber content is influenced by the addition of a binding agent (agar), which is a hydrocolloid source of polysaccharides that is recognized as fiber (Savitri, 2019).

The results of the Proximate Analysis were converted into the content of each product sheet, as shown in Table 2.

Table 2. Nutrient and micronutrient of the product sheet

Materials	Nutrient (g/12 g)			Micronutrient		
	Protein	Carbohydrate	Fiber	Vit C (mg / g)	Fe (mg / 100 g)	Antioxidant (%)
Spinach	0.822	4.17	4.21		3.31	
Yellow Pumpkins	0.895	4.77	3.84	0.53		
Purple Cabbage	0.442	5.19	4.36			56.21%

Notes: Nutritional requirements for children (g/day) of protein, carbohydrate, and fiber are 25, 220, and 20 for 4-6 y.o.; and 40, 250, and 23 for 7-9 y.o. (Permenkes No.28 Tahun 2019).

As shown in Tables 3 and 4, the nutrient content in one sheet of the product can only meet 2-3% of protein intake requirements, ±2% for carbohydrate intake, and 18-21% for fiber intake. This means that Edible Folding Paper cannot be used as a primary source of nutrition; other primary sources are still needed. Several sheets must be consumed to meet the daily intake requirements. However, Edible Folding Paper can be used as a product to help meet daily fiber intake.

Micronutrient Analysis

The analysis of iron content (Fe) in spinach Edible Folding Paper products resulted in 3.31 mg/100 g. Based on the recommended daily mineral intake according to the Minister of Health Regulation No. 28 (Kemenkes RI, 2020), children aged 4-12 years require an iron intake of 8-10 mg/day.

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Each sheet weighed 12 g, and the iron content per sheet was 0.397 g. Additional intake from other food sources is necessary to meet the recommended intakes.

In Edible Paper products made from purple cabbage, the analysis of antioxidant activity as an expression of the anthocyanin content in cabbage showed an inhibition value of 56.21%. This value indicates that the product has a high potential as a food source with high antioxidant activity.

The vitamin C content of the Edible Folding Paper yellow pumpkin products was 0.53 mg/g. Therefore, 12 g or 1 sheet of Edible Folding Paper contains 6.36 mg of vitamin C. This value, when referring to the recommended daily vitamin intake per person for the 4-12 age range according to Permenkes no.28 (Kemenkes, 2020), is 45–50 mg/day. This indicates that the Edible Folding Paper of Yellow Pumpkin has great potential as a complementary/additional source of vitamin C.

Fold Test

Edible Folding Paper products were tested for folding to determine their capabilities and durability as folding papers. The test results are listed in Table 3.

Table 3. Total fold of edible folding paper

Materials	Total fold
Spinach	201±5
Yellow Pumkins	205±6
Purple Cabbage	198±4

Note: Data (mean±SD) were obtained from three replicates.

The test results in Table 3 show that all variants of the Edible Folding Paper products have a folding value above 100, indicating that all product variants have excellent folding capabilities (Widiastuti et al., 2024). This data is further reinforced by the product application test results in Figure 2, where all product variants can be shaped into several types of origami.

The fiber content analysis of all product variants showed consistently high values, exceeding 5% per 100 g, thereby classifying the formulations as fiber-rich products. In edible films, high fiber content typically increases stiffness and tensile strength while reducing extensibility and foldability due to restricted polymer chain mobility (Müller et al., 2009). However, our folding tests revealed excellent foldability for all variants. This unexpected flexibility may be explained by the presence of naturally occurring sugars and other low-molecular-weight constituents in the fruit and vegetable ingredients, which act as internal plasticizers, increasing molecular mobility and counteracting the stiffening effect of the fiber fraction. Similar phenomena have been reported in edible film studies, where the addition of plasticizers, such as glycerol or sugar, markedly enhanced flexibility and elongation at break and improved foldability and mechanical performance despite the presence of reinforcing components (Janowicz et al., 2023; Wu et al., 2025).

Figure 2. Edible folding paper applied in the form of origami

This study is limited by its laboratory-scale processing, restricted batch replication, and the absence of sensory evaluation, shelf-life testing, and child-acceptability assessment. Future research should optimize the formulation and drying parameters to

improve nutrient retention, explore larger sheet formats to enhance nutrient delivery, and expand color variants using additional vegetables. Sensory testing and real-use trials with children, as well as shelf-life and microbiological safety evaluations, are strongly recommended to support product refinement and broader implementation.

CONCLUSION

The Edible Folding Paper demonstrated a meaningful nutrient profile for children aged 4–10 years, contributing 2–3% of the daily macronutrient needs per sheet and up to 20% of the daily fiber requirements. The pumpkin variant delivered the highest vitamin C contribution (14%), the purple cabbage variant showed strong antioxidant activity (~50% inhibition), and spinach contributed approximately 5% of the daily intake. All formulations exhibited excellent foldability and structural integrity during origami application, supporting their potential as both nutritious products and interactive learning media.

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THE EFFECT OF DRYING CONDITIONS USING A FOOD DEHYDRATOR ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF RED CAYENNE PEPPER POWDER (*Capsicum frutescent* L.)

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ABSTRACT

Red cayenne pepper (*Capsicum frutescens* L.) is an important horticultural commodity in Indonesia with high demand; however, its high moisture content makes it highly perishable. One way to extend its shelf life is to process it into powder through drying. Drying using a *food dehydrator* is considered more effective because it allows precise control of temperature and time, which affects the final product quality, including moisture content, color, and vitamin C, β-carotene, and capsaicin levels. This study aimed to determine the effects of different drying temperatures and durations using a *food dehydrator* on the physicochemical characteristics of red cayenne pepper powder. A Completely Randomized Factorial Design was used with two factors: drying temperature (50, 60, and 70°C) and drying time (16, 20, and 24 h), resulting in nine treatment combinations with three replicates. The parameters observed included moisture content, yield, color (L*, a*, and b*), vitamin C, β-carotene, and capsaicin content. The results showed that increasing temperature and drying time significantly decreased the moisture content, yield, and vitamin C content, while increasing the brightness and redness of the pepper powder. β-carotene content increased at moderate temperatures and decreased at higher temperatures, while the highest capsaicin content was found at 60 °C for 20 h (998.96 ppm or 16,083.33 SHU) and decreased at 70 °C. Overall, drying at 60 °C for 20 h produced red cayenne pepper powder with optimal physicochemical quality.

Keywords: red cayenne pepper, drying, food dehydrator, physicochemical characteristics

INTRODUCTION

People in Indonesia are known to be very fond of foods with strong flavors, especially spicy foods. Spicy flavors are commonly associated with chili peppers. In Indonesia, there are various types of chili peppers, including large, curly, bell, katokkon, and cayenne peppers (Fadhilatunnur et al., 2022).

Cayenne pepper (*Capsicum frutescens* L.) is an important commodity in Indonesia's agricultural sector because of its high demand. Cayenne pepper thrives in various regions of Indonesia. According to data from the Central Statistics Agency (BPS) in 2023, cayenne pepper production in Indonesia increased significantly. Bird's eye chili production reached 1,544,441 tons (Central Statistics Agency, 2024), with household consumption reaching 610,800 tons.

Red cayenne pepper has a high water content, causing it to rot or spoil easily, making it difficult to maintain freshness. Damage to red cayenne pepper can be influenced by internal and external factors originating from outside the pepper (Setyawibawa et al. 2018). This is exacerbated by the lack of post-harvest handling by farmers. Therefore, measures must be taken to maintain the quality of chili peppers. One such measure is drying the grain.

Drying is a process of simultaneous water vapor transfer that requires energy to evaporate the water contained in the material and transfer it from the surface of the material to the drying medium. Several drying methods can be applied to extend the shelf life of chili peppers, such as drying with sunlight, hot air, ovens, and food dehydrators. The use of a food dehydrator in the drying process has the advantage of being able to control the

temperature and drying time precisely, allowing for a more uniform and consistent drying process. Drying can be applied to cayenne pepper to produce a product in the form of chili powder.

Chili powder processing minimizes damage to the cayenne peppers. Cayenne pepper powder is more practical to store and has a longer shelf life than fresh chilies. Chili powder has a very fine particle size and low moisture content

This study aimed to determine the effects of temperature and drying time using a food dehydrator on the physicochemical characteristics of red chili powder. This study also provides information on optimal drying temperature and time.

MATERIALS AND METHODS

Materials

The material used in this study was red cayenne pepper obtained from the Segiri Samarinda traditional market in Indonesia. The chemicals used were absolute ethanol, distilled water, capsaicin standard, ascorbic acid, and β-carotene.

Experimental Design and Data Analysis

This study was conducted using a completely randomized factorial design with two factors. The first factor was drying temperature variation at 50, 60, and 70 °C, and the second factor was drying time at 16, 20, and 24 h. Based on these two factors, nine treatment combinations were obtained and repeated thrice.

The parameters analyzed in this chili powder were its physical and chemical properties. The physical properties parameters were water content, yield and color. The chemical properties parameters were vitamin C, β-carotene and capsaicin. The data obtained were analyzed using ANOVA, followed by Duncan's Multiple Range Test.

Research Procedures

The red cayenne peppers were separated from their stems, washed thoroughly, and weighed to make 100 g. The clean red cayenne peppers were dried using a food dehydrator according to the treatment, then blended and filtered using an 80-mesh sieve.

Preparation of Materials

The time used for drying red cayenne pepper was 16, 20, and 24 h, while the temperatures used were 50, 60, and 70 °C. A total of 100 g of sample was used for each treatment. The chemicals used for chemical analysis were absolute ethanol, distilled water, ascorbic acid, and capsaicin standard.

Analysis Procedure

The analysis procedure used in this study was the recommended moisture content (Murti, 2017), yield (Setyawibawa et al., 2018) and color (Rizquna et al., 2024). Vitamin C (Sihotang and Meilani, 2024), β-carotene (Hagos et al., 2022), and capsaicin (Amaliah, 2018).

RESULTS AND DISCUSSION

The drying conditions significantly affected (p<0.05) the yield and moisture content of red cayenne pepper powder, but not the color (Table 1).

Yield

Yield is an important parameter in the drying process that describes the efficiency of converting fresh materials into dry products. The yield value was obtained by comparing the final weight of the product after drying to the initial weight before drying (Mar'atuzzahwa et al., 2022).

In this study, the yield of red cayenne pepper powder showed significant differences between temperature treatments and drying times. The highest yield was obtained from the treatment at a temperature of 50 °C for 16 h, which was 49.44±0.39%, while the lowest yield was obtained at a temperature of 70 °C for 24 h, which was 14.63±0.24%.

The decrease in yield that occurs with increasing temperature and drying duration indicates that overly intensive drying processes can cause a significant reduction in the dry matter weight. This is due to excessive water evaporation and the loss of volatile components, such as essential oils and aromatic compounds that usually evaporate at high temperatures. This study is in line with the results of a study by Fadhilatunnur et al. (2022), who stated that drying at high temperatures or for too long can reduce the final yield of the material due to excessive

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evaporation and damage to volatile compounds.

Moisture Content

Moisture content is one of the main parameters for determining the quality of cayenne pepper powder. Moisture remaining in cayenne pepper powder can affect physical characteristics such as texture and color, as well as determine the product shelf life. High moisture content can increase the risk of microbial growth, such as bacteria, mold, and yeast, which accelerate the spoilage. The low moisture content in red chili powder indicates that the drying process is effective in reducing the water content and extending the shelf life of chili powder (Saputri et al., 2022).

Based on the results of the study, the highest moisture content was found in the 50 °C drying treatment for 16 h at 40.05%, and the lowest moisture content was found in the 70 °C drying treatment for 24 h at 1.75%. These results are consistent with those of Saputri et al. (2022). The decrease in water content occurs because some of the water contained in the cayenne pepper evaporates during the drying process, which utilizes heat energy (Jamilah et al., 2019). The higher the temperature used for drying, the greater the heat energy transferred, which accelerates and increases water evaporation. Accordingly, a longer drying duration correlates with greater water loss from the material. As a result, the moisture content in the cayenne pepper powder will be low (Citra et al., 2023)

Table 1. Effect of drying conditions on yield and physical characteristics of red cayenne pepper powder

Sample	Yield	Moisture Content	L	a	b	ΔE	Color Representation
Control	100±0,00	60,93±1,24	39.32	63.51	87.14		
50 °C, 16 h	49,44±0,39^{a}	40,05±1,73^{a}	47.08	23.76	39.62	61.59	
50 °C, 20 h	37,01±0,05^{b}	14,57±0,21^{b}	54.04	22.66	43.03	20.11	
50 °C, 24 h	22,59±0,45^{c}	12,92±0,60^{c}	51.99	21.72	42.93	59.66	
60 °C, 16 h	21,68±0,19^{d}	7,40±0,34^{d}	55.03	27.24	53.35	55.61	
60 °C, 20 h	20,23±0,24^{e}	5,40±0,39^{e}	58.20	25.38	52.64	52.54	
60 °C, 24 h	19,67±0,11^{f}	4,70±0,18^{e}	59.22	24.10	52.32	54.28	
70 °C, 16 h	17,72±0.14^{g}	3,54±0,20^{f}	58.20	25.38	52.64	52.44	
70 °C, 20 h	15,74±0.04^{h}	2,44±0,09^{fg}	59.60	26.05	52.73	54.66	
70 °C, 24 h	14,63±0.24^{i}	1,74±0,06^{g}	59.23	25.19	53.38	51.84	

Note: Data (mean ± standard deviation) were obtained from three replicates. Data were analyzed using analysis of variance (ANOVA). Data in the same column (excluding control) followed by different letters indicate significant differences (DMRT, $p<0.05$).

Color

Color analysis is an important parameter for determining the visual quality of a product, as it can affect the consumer perception of red chili powder. In this study, color measurements were performed using the CIE Lab* color system, which consists of three main components: L* (brightness level), a* (red-green), and b* (yellow-blue).

Based on the measurement results of L*, a*, and b* values at various combinations of temperature and drying time, there was a significant difference influenced by temperature variations. The L* value, which indicates the level of brightness, tended to increase with increasing temperatures from 50 to 70 °C. The highest value was obtained at 70 °C (59.60 ± 0.48), whereas the lowest value was found at 50 °C (47.08 ± 2.14). The a*

value, which represents the intensity of red color, also increased from 22.60±1.55 at 50°C to 25.53±1.20 at 70 °C, indicating a stronger red color at higher temperatures. Similarly, the b* value, which indicates the tendency of yellow color, increased from 41.76±2.34 at 50 °C to 48.64±5.98 at 70 °C, indicating a stronger dominance of yellow color at higher drying temperatures.

This study is in line with the research conducted by (Murti, 2017) which shows that drying at high temperatures has a significant effect on the color change of Lado F1 curly chili peppers. The results of this study show that the L* a* b* color values tended to increase as the temperature increased from 50 to 80 °C. The L* value increased from 30.20 to 31.17, the a* value from 17.77 to 27.93, and the b* value from 8.53 to 11.80, respectively. This increase in color values indicates that the dried chili products have a brighter and more attractive color appearance. This phenomenon is due to faster water evaporation and the inactivation of color-degrading enzymes, which allows natural pigments such as carotenoids to remain stable. In addition, the increase in L*, a*, and b* values was caused by the formation of a surface that was more reflective of light due to the decrease in water content, as well as better color pigment stability at high temperatures during the optimal drying duration (Murti, 2017).

Chemical Characteristics

The drying conditions significantly affected (p<0.05) all chemical components studied (vitamin C, β-carotene, and capsaicin) (Table 2).

Table 2. Effect of drying conditions on chemical characteristics of red chili powder

Sample	Vitamin C	β- Carotene	PPM	Scoville Heat Unit (SHU)
Control	1293.06±63.63	15.83±10.52	790.87±98.36	12,732.95±1,583.56
50 ˚C, 16 h	931.84±7.20^{a}	47.89±9.53^{b}	760.73±2.82^{de}	12,247.82±45.49^{de}
50 ˚C, 20 h	893.29±4.53^{ab}	108.29±11.77^{b}	683.52±18.16^{e}	11,004.70±292.40^{e}
50 ˚C, 24 h	867.21±13.24^{bc}	128.92±44.48^{b}	735.31±12.95^{e}	11,838.50±208.42^{e}
60 ˚C, 16 h	841.59±14.78^{c}	193.48±47.63^{a}	960.36±76.86^{ab}	15,461.76±1,237.47^{ab}
60 ˚C, 20 h	795.56±11.02^{d}	219.91±19.88^{a}	998.96±77.17^{a}	16,083.33±1,242.48^{a}
60 ˚C, 24 h	766.53±0.00^{de}	225.19±21.57^{a}	885.97±21.58^{bc}	14,246.12±347.36^{bc}
70 ˚C, 16 h	745.22±14.37^{ef}	236.58±46.97^{a}	844.54±43.54^{cd}	13,597.07±705.06^{cd}
70 ˚C, 20 h	708.71±20.44^{f}	230.90±40.67^{a}	736.56±64.60^{de}	12,293.30±1,040.10^{de}
70 ˚C, 24 h	595.56±67.45^{g}	215.94±43.34^{a}	698.59±56.28^{e}	11,247.26±906.19^{e}

Note: Data (mean ± standard deviation) were obtained from three replicates. Data were analyzed using analysis of variance (ANOVA). Data in the same column (excluding control) followed by different letters indicate significant differences (DMRT, p<0.05).

Vitamin C

Analysis of vitamin C content in dried red chili powder using a combination of temperature and time showed that vitamin C levels decreased significantly as the temperature increased and the drying time lengthened.

The highest vitamin C content was obtained at a temperature of 50 °C for 16 h (931.84 µg/g), and then gradually decreased to 867.21 µg/g at 24 h. This decline continued at a temperature of 60 °C and a drying time of 16 h, where the vitamin C content decreased to 841.59 µg/g, and further decreased to 766.53 µg/g at 24 h. The most significant decline occurred at 70 ˚C, where the lowest vitamin C content of 595.56 µg/g was observed.

These results indicate that vitamin C is easily degraded and highly sensitive to temperature and drying time. An increase in drying temperature accelerates the oxidation and decomposition of vitamin C, thereby reducing its content.

In addition, a longer drying time provides a greater opportunity for vitamin C

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degradation (Paramita, 2023). This study is in line with (Putranto, 2021) which states that high temperatures and drying time significantly reduce vitamin C levels in carrots because of the nature of vitamin C, which is easily damaged by heat.

β-Carotene

β-Carotene is a type of carotenoid compound commonly found in red, yellow, and orange vegetables and fruits. Based on the results of this study, the β-carotene content in red cayenne pepper powder decreased as the temperature and drying time increased.

At a temperature of 50 ˚C, the lowest β-carotene content recorded was 47.89 µg/g with a drying time of 16 h, and it increased to 128.92 µg/g at 24 h. When the temperature was increased to 60 °C, the β-carotene content increased further, from 193.48 µg/g (16 h) to 225.19 µg/g (24 h). The most significant decrease occurred at 70 ˚C, with a β-carotene content of 236.58 µg/g at 16 h and reaching a low point of 190.02 µg/g at 24 h of drying.

The significant increase in β-carotene content during the drying process is closely related to the decrease in moisture content and the change in the basis of calculation from a wet to a dry basis. Under control conditions, where the moisture content is still high, β-carotene is measured on a wet basis, causing the pigment to be dispersed or diluted in a large mass of water, resulting in low concentrations.

As the drying process progresses, the water content decreases significantly, such that the calculation of nutrient content proportionally better reflects the dry basis conditions. Under these conditions, the lost water mass did not contribute to the total mass of the material. Consequently, although the absolute amount of β-carotene did not increase, its relative concentration increased because the total mass of the material decreased. This decrease in water mass reduces the divisor value in the calculation, that is, dry mass + water mass. The lower the water content, the smaller the divisor value, so that the ratio of β-carotene per unit mass of material increases, resulting in a much higher concentration (Prastikasari et al., 2023).

This phenomenon creates a clear negative relationship between water content and β-carotene, where progressive water evaporation reduces the divisor, causing the pigment concentration to surge. The exponential decrease in water content increases the specific dry weight proportion, causing the ratio of β-carotene per gram of dry weight to increase significantly because the measurement divider remains relatively stable while the diluting water is removed. This explains the sharp increase in β-carotene content during the early to middle stages of drying, where the increase in dry weight is directly correlated with pigment concentration (Histifarina et al., 2004).

At temperatures above 70 ˚C, the decrease in β-carotene content with increasing temperature and drying time is caused by higher heat energy triggering a faster degradation process through oxidation and isomerization, which damages the polyene chain of β-carotene and changes its form from trans to the less stable cis. This process causes a significant decrease in β-carotene content, especially after prolonged heating, because chemical damage to the pigment is more dominant than tissue release. As a result, volatile compounds such as aldehydes and ketones are formed, which affect the reduction in red color intensity, nutritional value, and product stability (Pratita et al., 2023).

The degradation of β-carotene can occur due to the presence of lipoxygenase enzymes, which are inactivated by heating. The results of this study are in line with those of Halimah et al. (2021), who showed that the β-carotene content in pineapple juice decreased significantly as the pasteurization temperature increased. At 70 °C, the β-carotene content was recorded as the highest, whereas at 90 °C, there was a fairly drastic decrease in content.

β-Carotene is a carotenoid pigment that gives foodstuffs an orange to reddish color. Degradation caused by β-carotene content results in a decrease in the color intensity of chili powder, making it paler or faded in color. This can be observed from the color analysis results (L* a* and b* values), where the higher the temperature and drying time, the more the red color intensity decreases (Meriska, 2022).

Capsaicin

The capsaicin content in cayenne pepper powder is greatly influenced by temperature and drying time, which also directly affect the Scoville Heat Unit (SHU) value as an indicator of spiciness. Based on the results of this study, temperature and drying time affect the capsaicin content in red cayenne pepper powder.

The data obtained showed that at a temperature of 50 °C with a drying time of 16 h, the capsaicin content was 760.73±2.82 ppm with an SHU of 12,247.82±45.49. Then, it decreased at 20 h to 683.52±18.16 with an SHU value of 11,004.70±292.40 and increased again at 24 h to 735.31±12.95 with an SHU value of 11,838.50±208.42. At 60 °C, the capsaicin content increased further to 960.36±76.86 ppm, with an SHU value of 15,461.76±1,237.47. The highest capsaicin content was obtained at 20 h with a value of 998.96±77.17 ppm and an SHU value of 16, whereas at 70 °C, the capsaicin content tended to decrease with values of 844.54±43.54 ppm and 13,597.12±347.36 SHU. At 70 ˚C, the capsaicin content tended to decrease with successive values of 844.54±43.54 ppm and an SHU value of 13,597.07±705.06, 736.56±64.60 ppm and an SHU value of 12,293.30±1,040.10, 698.59±56.28 ppm and an SHU value of 11,247.26±906.19.

The capsaicin content in fresh red chili peppers tends to be low compared to 50 ˚C and 60 ˚C. This is because lower temperatures are not optimal for the maximal release of capsaicin from the chili tissue. At these temperatures, the drying process occurs more slowly; therefore, the moisture content in the material does not decrease significantly, causing capsaicin to remain trapped in the cell matrix and not be measured to its fullest extent. This is consistent with the findings of Khairani et al. (2024), who stated that a decrease in water content plays an important role in increasing capsaicin yield because the lower the water content, the more concentrated the active compounds are in the dry material. Conversely, a temperature of 60 °C provides greater heat energy to accelerate water evaporation and help release capsaicin from the tissue, but is still within the safe limits for capsaicin thermal degradation. Similar research was also described by Rachmat et al. (2023), who showed that the decrease in water content during the drying process plays an important role in increasing the spiciness level of chili powder. High water content causes a lower capsaicin concentration, resulting in a final product with suboptimal spiciness. However, at 70 °C, the capsaicin content decreased compared to that at 60 °C. This decrease indicates that 70 °C exceeds the thermal stability limit of capsaicin, triggering its degradation of the capsaicin compound. Increasing the temperature and drying time will cause a decreases the spiciness of chili peppers, as capsaicin is highly sensitive to heat. This degradation is caused by damage to the chemical structure of capsaicin due to excessive heat, which ultimately reduces the concentration of active compounds in the final product (Hayati et al., 2023).

CONCLUSION

The combination of drying temperature and time significantly affects the physicochemical characteristics of red cayenne pepper powder. The best treatment was obtained at a temperature of 60°C for 20 h, with a moisture content of 5.40%, yield of 20.23%, color ΔE of 52.54, vitamin C of 795.56 μg/g, β-carotene of 219.91 μg/g, and the highest capsaicin content of 998.96 ppm (16,083.33 SHU). A temperature of 60°C is optimal because it provides balanced heat energy to effectively reduce moisture content, maintain moderate yield without excessive loss of volatile compounds, produce attractive bright colors, and maintain the stability of vitamin C and β-carotene through adequate water evaporation without excessive oxidation as at 70°C. while the 20-hour duration achieves peak capsaicin concentration with the release of active compounds from the cell matrix before thermal degradation occurs at longer durations, thereby producing the best physicochemical quality overall.

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PHYSICAL AND SENSORY CHARACTERISTICS OF SOURDOUGH BREAD ENRICHED WITH BEETROOT (*Beta vulgaris* L.) POWDER

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ABSTRACT

This study investigated the effect of beetroot powder incorporation on the physicochemical, textural, and sensory properties of sourdough bread as part of efforts to develop nutritionally enhanced bakery products with acceptable quality. Four formulations of composite flour of 300 g were prepared: a control (without beetroot powder) and sourdough breads substituion of beetroot powder of 5, 10, and 15%. The physicochemical and textural characteristics were evaluated using texture profile analysis, including hardness, cohesiveness, gumminess, chewiness, adhesiveness, stiffness, and springiness, while sensory attributes were assessed based on appearance, aroma, taste, texture, and overall acceptability. The results demonstrated that increasing beetroot powder levels significantly affected most textural parameters. The highest values of hardness (254.79 gf), gumminess (57.75), and chewiness (4.51) were observed in bread with substitutuion with beetroot powder of 15%, indicating a denser crumb structure at 15% substitution level. Cohesiveness and stiffness increased with beetroot addition, whereas springiness generally decreased, suggesting a trade-off between structural rigidity and elasticity of the dough. Sensory evaluation revealed that the control bread achieved higher scores across most attributes; however, the bread with beetroot powder substitution of 10% recorded the highest overall acceptability among the beetroot-enriched breads (3.90 ± 0.84). Moderate beetroot incorporation enhanced the visual appeal and flavor balance without masking the characteristic of the sourdough. However, excessive beetroot substitution negatively affected sensory quality due to the dominant earthy flavor and less desirable texture. In conclusion, 10% beetroot powder substitution provides an optimal balance between nutritional enhancement, textural quality, and consumer acceptance, offering valuable insights for the formulation of functional sourdough bread products.

Keywords: beetroot powder, functional bakery products, sensory evaluation, sourdough bread, physical analysis

INTRODUCTION

Indonesia is renowned for its rich biodiversity, particularly in the production of tropical fruits; however, beetroot (*Beta vulgaris* L.) stands out because of its unique nutritional benefits and potential applications in food processing. The health implications of beetroot consumption are significant. As indicated in multiple studies, beetroot is a powerhouse of nutrients, including essential vitamins such as vitamin C and minerals, including iron and calcium (Amelia et al., 2024). Its bioactive compounds, particularly betalains and nitrates, contribute significantly to health benefits, such as lowering blood pressure, enhancing physical performance, and reducing oxidative stress (Amelia et al., 2024; Mirmiran et al., 2020). The antioxidant properties of beetroot, attributed to its high phenolic and carotenoid content, further underscore its importance in preventing non-communicable diseases (Liu et al., 2022).

Despite these benefits, beetroot remains underutilized in various culinary applications, particularly in regions such as Indonesia, where its culinary uses have yet to reach their full potential. One avenue for expanding beetroot usage is its incorporation into food products, such as sourdough bread. Beetroot flour, derived from dried beetroot, offers an innovative way to enhance the nutritional profiles of baked goods. The natural colorants and potential health benefits of dietary nitrates found in beetroot flour can

improve not only the appearance but also the overall health profile of bread products (Constantin et al., 2025).

Sourdough bread presents several distinct health benefits compared to industrial bread made using instant yeast. A core advantage of sourdough bread lies in its fermentation process, which fundamentally alters its nutritional composition. Sourdough fermentation involves a symbiotic relationship between lactic acid bacteria (LAB) and wild yeasts, which enhances nutrient bioavailability. Studies have indicated that sourdough fermentation can reduce anti-nutritional factors, such as phytic acid, thereby potentially increasing mineral availability in bread (D'Amico et al., 2023; Ribet et al., 2023). Furthermore, sourdough bread has been shown to lower the glycemic response due to the fermentation process, which alters the starch structure (D'Amico et al., 2023; Ribet et al., 2023).

The incorporation of beetroot powder into sourdough bread formulations offers several benefits. Beetroot powder is rich in nutrients, particularly nitrates, antioxidants, and dietary fiber, which enhance the health-promoting properties of sourdough bread. Nitrates from beetroot powder have been shown to improve vascular function and lower blood pressure, potentially conferring additional cardiovascular benefits (Akamine et al., 2023). Furthermore, the earthy sweetness of beetroot can improve the sensory profile of bread, providing an attractive color and taste without the need for added sugars (Alkay et al., 2024). The addition of beetroot powder can also increase the fiber content of bread, supporting better digestive health (Koistinen et al., 2018; Lau et al., 2021). Incorporating beetroot powder into sourdough bread has the potential to enhance not only the visual appeal through vibrant color but also the nutritional benefits due to the natural compounds found in beetroot. Thus, assessing the texture and sensory properties of food is fundamental for predicting consumer acceptance.

Given these considerations, this study aimed to analyze the physical and sensory characteristics of sourdough bread incorporated with beetroot powder. We hypothesized that the incorporation of beetroot powder would positively influence the antioxidant content, texture, volume development, and overall sensory acceptability of the bread. The novelty of this research lies in the integration of a functional, nutrient-rich, and underutilized ingredient into a traditional fermentation-based bread system. This approach contributes to food innovation that aligns with health trends and promotes the value of local biodiversity in the development of functional bakery products.

MATERIALS AND METHODS

Materials

The ingredients used in this study were those used to make sourdough bread, including flour (Bogasari Cakra Kembar brand), sugar (Gulaku Premium brand), sourdough starter (made by the researcher using natural yeast fermented from salak pondoh fruit (Rahardjo and Sihombing, 2023), water (Aqua brand), salt (Dolphin brand), and butter (Anchor unsalted butter).

Experimental Design and Data Analysis

This study was conducted at the Food Processing Laboratory of the Faculty of Health Sciences, Salatiga, from February to March 2025. The methods used in this research were divided into several steps, starting from sourdough bread making, texture analysis, sensory analysis, and data analysis.

Sourdough bread making

The white bread recipe was adapted from Forkish (2022), with the sourdough starter used as a substitute for instant yeast and the incorporation of beet powder in the formulation. The sourdough bread formulations were water 100 g, salt 3 g, butter 15 g, and sourdough starter 50 g, while a 300 g composite flour of wheat and beetroot powder was added. The beetroot powder was substituted in the 300 g wheat flour with a 0, 15, 30 and 45 g, which equivalen to 0, 5, 10, and 15%.

The dry and wet ingredients were first weighed and placed in separate large plastic containers. The two ingredients were then mixed, and the dough was kneaded until smooth by checking the window pane. The dough that has passed the windowpane check

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is left for bulk fermentation for 2.5-3.0 h, then weighed and cut to adjust to the size of the pan, proofing to the size of the pan height, and finally baked for 25-30 minutes until the surface of the bread is golden brown. Once baked, the bread was analyzed.

Physical analysis

Physical analysis included texture analysis performed using a Texture Analyzer (Lloyd TA Plus) in accordance with the procedure described by Kulthe et al. (2014), where the resulting analysis results were hardness, cohesiveness, springiness, gumminess, chewiness, fracture force, adhesive force, adhesiveness, and stiffness. Each sample was analyzed thrice.

Sensory analysis

Sensory analysis was performed using the acceptance test of preference rating (Meilgaard et al., 2016) of five parameters: appearance, aroma, taste, texture, and overall acceptance. The rating used is a rating with 5 scales (scale 1 is the least preferred and scale 5 is the most preferred). The panelists used for the sensory test were 50 untrained panelists.

Data analysis

All data obtained were analyzed using analysis of variance (ANOVA), then continued with Duncan's Multiple Range Test at $\alpha = 5\%$ to determine the treatment level that provides a real difference. All statistical tests were performed using IBM SPSS Statistics 29 software.

RESULTS AND DISCUSSION

Physical Analysis

The physical analysis of bread texture is essential in both academic research and industrial applications because it provides an objective evaluation of the quality attributes that directly influence consumer acceptance and shelf life. Textural properties, such as hardness, chewiness, springiness, and cohesiveness, serve as indicators of a bread's structural integrity and overall palatability (Wang et al., 2025). The results of the physical analysis in this study are presented in Table 1.

Table 1. Effects of beetroot powder substitution on physical characteristics of breads

Beetroot powder (g)	Hardness (gf)	Cohesiveness	Springiness (mm)	Springiness Index	Gumminess (N)	Chewiness (kgf.mm)	Adhesiveness (N.cm)	Stiffness (gf/cm)
0	129.19±12,25^{A}	0,05±0,017^{A}	12,29±0,30^{C}	1,55±0,12^{B}	6,92±2,36^{A}	0,83±0,27^{A}	0,01±0,01^{A}	0,02±0,00^{A}
15	152.85±79,34^{B}	0,12±0,04^{B}	5,53±2,36^{A}	0,51±0,09^{A}	25,95±15,56^{B}	1,63±1,50^{B}	0,03±0,00^{B}	0,04±0,00^{B}
30	177.96±8,70^{B}	0,12±0,04^{B}	7,82±2,20^{B}	0,54±0,21^{A}	30,33±6,70^{B}	1,67±0,29^{B}	0,05±0,00^{C}	0,02±0,00^{A}
45	254.79±23,09^{C}	0,23±0,08^{C}	7,97±0,73^{C}	0,55±0,04^{A}	57,75±8,87^{C}	4,51±0,75^{C}	0,02±0,00^{A,B}	0,03±0,00^{A,B}

Note: Data (mean±SD) were derived from three replications. The data were analyzed by ANOVA. The data within the same column followed by different letters showed significant difference (DMRT, $p<0,05$). The beetroot powder was added in a 300 g composite flour system of wheat and beetroot.

Hardness is a crucial parameter in the texture analysis of bread that directly correlates with sensory perception and consumer acceptance. It typically refers to the force required to compress or bite through the bread, which affects the overall mouthfeel and enjoyment of the product. The importance of analyzing hardness lies in its ability to provide insights into the quality and freshness of bread, as greater hardness is often associated with staling and reduced palatability (Sahin et al., 2024). Regarding the incorporation of beetroot powder in sourdough bread, studies have indicated that the addition of beetroot powder can result in increased hardness when included in higher amounts. In this study, sourdough bread formulations containing 15% beetroot powder exhibited the highest hardness values. This could be due to several factors, including the impact of beetroot powder on dough structure, moisture content, and interactions with starch and gluten (Ani et al., 2022). Moreover, while the inclusion of sourdough typically mitigates hardness, the contrasting effects of a substantial addition of beetroot

powder can improve the structural integrity of the bread, thereby increasing its overall hardness compared to the sourdough bread control (Škrobot et al., 2022; Wolter et al., 2014).

Further exploration indicated that when the percentage of beetroot powder exceeded the 15% threshold, the resulting hardness increased further. This can be attributed to the higher concentrations of beetroot powder, which led to significant changes in the moisture content and gluten structure within the dough, resulting in a denser and firmer crumb structure (Roby et al., 2020). The addition of various components, such as beetroot powder, directly influences the rheological properties of the dough, which ultimately plays a role in determining the textural attributes of the final product (Škrobot et al., 2022).

Cohesiveness in texture analysis, particularly in bread, refers to the ability of a material to maintain its structure during deformation, reflecting the internal interactions among the components of the bread matrix. It is quantitatively assessed through texture analysis as the ratio of the area under the stress-strain curve during the second compression cycle to that of the first (Mondal and Eun, 2023). Cohesiveness is important because it correlates with the perceived freshness and quality of the bread; lower cohesiveness often indicates that the bread is more prone to crumbling or disintegration during consumption (Matos and Rosell, 2012).

In this study, the addition of beetroot powder significantly affected the cohesiveness. Sourdough bread without such incorporation generally exhibits lower cohesiveness due to the basic yeast-fermented dough structure, which may not retain moisture and structural integrity as effectively as formulations enriched with other components, such as beetroot powder. The presence of beetroot powder, especially at a moderate incorporation level of approximately 15%, tends to enhance the gluten network or create new interactions within the dough matrix, leading to increased cohesiveness (Ani et al., 2022).

Springiness refers to the ability of the bread crumb to recover its shape after deformation, which is a measure of the elasticity of the bread texture. This is a significant characteristic of texture analysis, as it correlates with the perceived freshness and quality of the bread. A higher springiness value typically indicates a more desirable and elastic texture that consumers associate with freshness and quality (Matos and Rosell, 2012). The springiness index, often quantified through texture profile analysis (TPA), provides a numerical representation of this property, enabling standardized comparisons of different bread formulations (Alkay et al. 2022).

In the context of sourdough bread, the texture and quality can vary significantly depending on the ingredients. The results of this study indicate that the sourdough bread control typically exhibits a higher springiness index than those containing beetroot powder. This difference can be attributed to the strength of the gluten network, which is often compromised when beetroot powder is added (Aringalayan et al. 2022). The resulting bread may exhibit decreased elasticity and an increased rate of starch retrogradation, negatively impacting textural properties such as springiness (Nasehi et al., 2018).

The incorporation of beetroot powder may initially result in similar or slightly enhanced springiness at lower concentrations; however, increased concentrations can alter the dough matrix, leading to higher water absorption while weakening the gluten framework, which adversely affects the structure. Consequently, springiness decreased as the proportion of beetroot powder increased, leading to a more rigid crumb structure lacking desirable elasticity (Boz, 2015; Ranawana et al., 2016). Nonetheless, some findings suggest that within certain limits, beetroot inclusion can positively affect moisture retention and perceived springiness, particularly when balanced with other formulation factors (Begum et al., 2020).

Gumminess is an essential parameter for analyzing the texture of bread, especially sourdough. It combines elements of cohesiveness and hardness and indicates the energy required to chew a product thoroughly before swallowing. This texture attribute is critical for consumer acceptance and

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influences the overall quality perception of bread, as it affects the mouthfeel and initial bite. High gumminess can indicate undesirable textural qualities in bread, such as excessive chewiness and density, which can detract from the consumer experience.

In this study, we observed that the incorporation of beetroot powder significantly affected the gumminess measurement. Specifically, sourdough bread without beetroot powder typically exhibits lower gumminess than its beetroot-enhanced counterparts. This difference can be attributed to the structural changes induced by the addition of beetroot powder, which influences the water retention and gluten matrix interactions of the dough. The introduction of beetroot powder into the formulation interacts with the gluten matrix of the dough, leading to increased water binding capacity and improved dough elasticity. These interactions can enhance the cohesiveness of the bread crumb, contributing to a higher gumminess value than that of traditional sourdough formulations.

Moreover, as the percentage of beetroot powder in the bread increased, the gumminess values also increased. This can be explained by the dual mechanisms of enhanced water retention and the formation of a thicker gel-like structure within the bread matrix. Higher concentrations of beetroot powder contribute to more sugar and fiber and promote better hydration of starches and proteins, leading to a more uniform crumb structure that resists crumbling and enhances gumminess. Additionally, the interaction of soluble fibers from beetroot with starch can lead to modified retrogradation behavior during cooling, further positively influencing the bread texture.

Chewiness is a critical textural attribute of bread that significantly influences the overall sensory experience and palatability (Dunteman and Lee, 2023; Tian et al., 2024). It is characterized by resistance to biting and the effort required to chew, contributing to the mouthfeel and enjoyment of the eating experience. A finer balance in chewiness is particularly important in sourdough bread, where texture plays a role in consumer acceptance and may interact with nutritional and metabolic aspects (Nouska et al., 2023).

In this study, the control sourdough bread typically exhibited lower chewiness values than the formulations that incorporated beetroot powder. This difference can be attributed to the unique interactions between sourdough ferments and the structural properties of the bread matrix created during fermentation. It has been established that sourdough fermentation processes can lead to variations in chewiness due to the breakdown of gluten structures and alterations in starch behavior (Terrazas-Avila et al., 2024). Microbial activity and fermentation duration impact these interactions, often resulting in softer bread with reduced chewiness (Naji-Tabasi et al., 2023).

In contrast, incorporating beetroot powder into sourdough bread formulations can enhance the chewiness characteristics. This phenomenon can be explained by the influence of dietary fibers and bioactive compounds present in beetroot powder, which modify the rheological properties of the dough. As the percentage of beetroot powder increased, the hydration of the dough matrix also increased, leading to more cohesive and elastic properties that enhanced chewiness (Rizzello et al., 2012; Tian et al., 2024). The balance between the additional fiber content and moisture retention achieved through beetroot powder is essential, as it can counteract the typical softening of the crumb associated with standard sourdough bread (Zhang et al., 2012). Moreover, studies have indicated that higher levels of dietary fiber, such as those found in beetroot, contribute to a denser crumb structure, which often results in increased chewiness due to reduced gas bubbles during fermentation (Škrobot et al., 2022).

In analyzing the importance of adhesiveness within the context of bread texture, it is crucial to recognize that adhesiveness plays a vital role in the final sensory attributes of bread, influencing consumer acceptance and the overall quality of the bread. Adhesiveness, a texture parameter, refers to the degree of stickiness of the bread, which affects the mouthfeel and can impact mechanical handling during

production, ultimately influencing the quality and consumer satisfaction with the product (Raczyk et al., 2022).

The adhesiveness of sourdough bread without beetroot powder tended to be lower than that with beetroot powder. This is primarily because traditional sourdoughs are based on gluten, which contributes to a cohesive and elastic dough, reducing stickiness. In contrast, the addition of beetroot powder modifies the gluten network, introducing additional water-soluble compounds and fibers that alter the rheological properties of the dough (Ani et al., 2022; Raczyk et al., 2022). Consequently, the presence of beetroot powder can lead to an increase in moisture retention and a change in structure, which can enhance the adhesive qualities of the bread, making it feel stickier upon consumption.

Interestingly, the increase in the percentage of beetroot powder further increased the adhesiveness of the bread. This phenomenon can be attributed to several factors such as the following. First, increased beetroot powder adds more soluble fiber and polysaccharides to the dough, which enhances moisture retention and, consequently, increases adhesion properties. Beetroot fibers are known to form hydrophilic interactions with water molecules, thereby increasing the pliability and stickiness of the bread structure (Ani et al., 2022). Additionally, an excess of beetroot powder alters starch gelatinization and fiber matrix formation during baking, leading to a softer, more adhesive crumb texture (Clifford et al., 2015). High fiber content is associated with greater water absorption capabilities, which likely increases the observed adhesiveness during texture analysis (Hobbs et al., 2012; Preethi et al., 2020).

However, in this study, the situation markedly changed when the beetroot powder concentration in the sourdough bread formulation reached approximately 15%, indicating that a decrease in adhesiveness was noted at this threshold. This unexpected reduction in adhesiveness may be due to the interaction of beetroot components with the gluten network within the bread. As the beetroot concentration increases, the gluten structure may become compromised due to the competing hydrophilic properties of beetroot constituents, thereby reducing the stickiness of the dough (Ranawana et al., 2016; Vasconcellos et al., 2016).

In the context of texture analysis of bread, stiffness refers to the resistance of the bread crumb to deformation under an applied force. It is characterized by the maximum force exerted during compression tests, where higher values typically indicate firmer crumb structures. This study indicates that the sourdough bread control generally exhibited lower stiffness than the bread formulations incorporating beetroot powder. The incorporation of beetroot powder alters the physicochemical properties of the dough, leading to changes in water absorption and gluten matrix interactions, which can ultimately affect the stiffness of the final bread product (Adamczyk et al., 2021; Ani et al., 2022). However, as the percentage of beetroot powder in the formulation increased, the stiffness increased, contrary to the initial decrease at lower concentrations (such as 10%). This behavior can be attributed to changes in moisture content, structure, and interactions between starch and fiber components introduced by beetroot powder.

However, when the concentration is adjusted to higher levels, the additional beetroot powder can lead to a firmer crumb due to elevated fiber content or changes in the hydration properties, which enhances gluten development and contributes to a more structured matrix, thus increasing stiffness again at higher concentrations (e.g., 15%) (Ani et al. 2022). This nonlinear response (initial decrease in stiffness at 10% followed by an increase at 15%) highlights the complexity of ingredient interactions in bread formulation and demonstrates the importance of optimizing component ratios to achieve the desired textural characteristics. Moreover, the unique properties of beetroot powder, such as its high dietary fiber content, can significantly modify the structure and texture of the bread crumb, thereby impacting consumer acceptance and sensory outcomes (Ani et al., 2022).

Sensory Analysis

Sensory analysis is crucial in bread research and journal writing because it

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provides a comprehensive evaluation of a product's overall quality, encompassing flavor, aroma, texture, and appearance, attributes that may not be fully captured by chemical or instrumental methods alone. Sensory methods can account for human perceptions, such as flavor intensity and overall acceptability, thus offering a more holistic view of consumer experience (Elía, 2011). The results of the sensory analysis are presented in Table 2.

Table 2. Effects of beetroot powder substitution on sensory characteristics of breads

Beetroot powder (g)	Appearance	Aroma	Taste	Texture	*Overall*
0	4.50±0.68^{C}	3.90±0.84^{C}	4.14±0.88^{C}	3.86±0.81^{B}	4.16±0.77^{C}
15	3.78±0.93^{A}	3.60±0.90^{A}	4.00±0.93^{B}	3.92±0.83^{B}	3.82±0.83^{B}
30	3.80±0.97$^{A, B}$	3.78±0.89^{B}	4.14±0.86^{C}	3.96±0.83^{B}	3.90±0.84$^{B, C}$
45	3.86±1.01^{B}	3.76±0.94^{B}	3.12±1.04^{A}	3.66±0.89^{A}	3.74±0.94^{A}

Note: Data (mean±SD) were derived from 150 sensory responses. The data were analyzed by ANOVA. The data within the same column followed by different letters showed significant difference (DMRT, p<0,05). The beetroot powder was added in a 300 g composite flour system of wheat and beetroot.

In sensory analysis, the appearance of bread plays a crucial role, as it directly influences consumer acceptance and overall perception of quality. Studies emphasize that sensory attributes, including appearance, significantly affect the acceptability of various bread types, as consumers often rely on visual characteristics, such as color and shape, before making a purchasing decision (Callejo, 2011). Appearance not only dictates initial impressions but also informs consumers about taste and quality expectations (Sahin et al., 2024).

In this study, it was observed that the sourdough bread control (without additional ingredients such as beetroot powder) received higher appearance ratings than those incorporated with beetroot powder. This may be attributed to the color and visual texture modifications imparted by the food coloring effect of beetroot. Although beetroot has nutritional benefits, it can alter the perceived freshness and wholesomeness of bread, potentially lowering its sensory appeal (Hernández-Figueroa et al., 2024; Katsi et al., 2021). The traditional visual characteristics associated with sourdough bread, including crust coloration and crumb structure, are often perceived as more desirable by consumers, leading to higher ratings for the control sample (Katsi et al., 2021).

Interestingly, as the percentage of beetroot powder in the bread formulation increased, the appearance rating also increased. This phenomenon can result from the enhanced color depth and visual contrast provided by beetroot powder. Vivid coloration may attract consumers by presenting a unique and innovative product, thus increasing its aesthetic appeal (Nasir et al., 2020; Sahin et al., 2024). Additionally, incorporating vegetable powders, such as beetroot, can generate curiosity, particularly among health-conscious consumers who appreciate the added nutritional value. However, to balance this appeal with sensory attributes such as texture and aroma, it is essential that the percentage of beetroot does not compromise other critical factors that contribute to the overall sensory profile of the bread (Sahin et al., 2024).

Aroma plays a crucial role in the sensory analysis of bread, influencing consumer acceptance and enjoyment. In the context of bread, particularly sourdough, aroma arises from a complex interplay of fermentation, Maillard reactions, and the biochemical activity of yeast and lactic acid bacteria (LAB) during leavening (Aslankoohi et al., 2016; Winters et al., 2019; Zolfaghari et al., 2017). These biochemical processes generate a bouquet of volatile compounds, including alcohols, esters, and aldehydes, which contribute to the characteristic flavor profiles that consumers expect from high-quality bread (Heitmann et al., 2017; Mietton et al., 2022). Moreover, studies have shown that a desirable aroma profile may enhance

the overall sensory acceptance of a product, as the complexity of aroma compounds can elevate the perception of freshness and quality in baked goods (Callejo, 2011; Paravisini et al., 2019).

When comparing the aroma profiles of sourdough bread to those of sourdough incorporated with beetroot powder, the fundamental difference lies in the presence and ratios of aromatic compounds produced during fermentation and subsequent baking. Typically, sourdough bread exhibits a stronger and more appealing aroma owing to the fermentation process, which produces more volatile compounds, such as hexanal and 2-nonenal, that are associated with positive sensory evaluations (Birch et al., 2013; Katsi et al., 2021). In contrast, the incorporation of beetroot powder, particularly at lower concentrations, can introduce different aroma profiles that may be less acceptable to consumers. However, it has been observed that at moderate levels, beetroot powder can add complexity to the aroma without overpowering it. This preference might be linked to the balance of sweetness and earthy notes introduced by beetroot, which can complement the traditional sourdough aroma (Corona et al., 2016; Moskowitz et al., 2012).

The observation that a higher percentage of beetroot powder initially enhanced aroma ratings, while a threshold (approximately 15%) caused a decline in rating values, can be explained through sensory perception theory and the saturation point of aromatic compounds. Lower concentrations of beetroot powder may result in a pleasant additive effect, where the earthy sweetness of beetroot harmonizes with the characteristic smell of sourdough (Paravisini et al., 2019; Pico et al., 2017). However, exceeding the optimal concentration may overpower the inherent aromas of sourdough with excessively earthy and vegetal notes, detracting from the overall complexity appreciated by consumers (Haque et al., 2020; Suha et al., 2021).

Taste parameters in the sensory analysis of bread are critical for understanding consumer preferences and product acceptability. It encompasses various attributes, including sweetness, sourness, bitterness, and saltiness, which contribute to the overall flavor profile of bread products. This parameter is particularly important in sourdough bread, as the fermentation process significantly influences these sensory attributes owing to the activity of lactic acid bacteria (LAB) and yeast. LAB produce organic acids during fermentation, contributing to the sour taste characteristic of sourdough bread while enhancing its texture and nutritional quality (Zhao and Gänzle, 2016). Consumer preferences often favor bread varieties with distinct tastes that align with personal preferences, making the tasting component paramount for product development (Škrobot et al., 2022; Supasil et al., 2022).

When exploring why the taste rating of conventional sourdough bread was higher than that of sourdough bread incorporated with 5% and 15% beetroot powder, it is essential to consider the impact of beetroot's earthy flavor and sweetness. Studies have shown that the addition of beetroot powder may introduce off-flavors or distract from the typical sour notes expected in sourdough, potentially lowering the overall taste (Harth et al., 2018). Moreover, while 10% beetroot powder incorporation harmonizes well with the traditional sourdough flavor, with no significant departure from the sourdough bread control taste profile, higher concentrations likely overshadow the sour notes through excessive sweetness or earthy flavors, thereby diminishing consumer preference.

The unique case of 10% beetroot powder, which reflected taste ratings similar to those of traditional sourdough, can be attributed to a balance in which the added sweetness complements rather than overwhelms the sour flavors contributed by LAB fermentation. This balance showcases how specific ingredient levels can affect sensory properties while maintaining desirable sourdough characteristics (Hanis-Syazwani et al., 2018). Conversely, the inconsistent results with the 15% incorporation level may indicate that at this ratio, the earthy and sweet flavors from the beetroot become overwhelming, leading to lower product acceptance. This trend has been observed in other studies, where the

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excessive incorporation of certain ingredients negatively impacted sensory characteristics (Roby et al., 2020).

In sensory analysis, the texture of bread is a vital attribute that influences consumer preferences and overall acceptability. It encompasses various measurable qualities, such as hardness, chewiness, cohesiveness, and springiness, which collectively determine the mouthfeel and enjoyment of the product. The texture of bread significantly affects its quality and consumer appeal, as it is linked to the freshness, internal structure, and mechanical properties of the crumb, which are influenced by fermentation and baking conditions (Sidari et al., 2020).

When comparing sourdough bread with varying concentrations of beetroot powder, it was observed that bread with 5% and 10% beetroot exhibited higher texture parameter values than traditional sourdough bread without additives. This can be attributed to the interplay between the gluten network and non-gluten components introduced by beetroot powder. The addition of these powders influences moisture retention and elasticity, softening the texture when used within these limits (Ani et al., 2022).

However, when 15% beetroot powder was incorporated into the formulation, the texture parameter values were the lowest. This adverse effect occurred due to excessive amounts of beetroot powder, leading to the disruption of the gluten matrix necessary for maintaining the structure of the bread. A saturation point is reached where microbial and enzymatic activities essential for proper fermentation are hindered, resulting in a less cohesive and more fragile crumb structure (Popescu et al., 2022; Raczyk et al., 2022). This suggests a negative impact on the textural integrity of bread, as high concentrations dilute the effectiveness of gluten, which is crucial for texture.

In sensory analysis, overall acceptability is a critical parameter that reflects consumers' perceptions of a product based on a combination of attributes, including taste, aroma, texture, and visual appeal. This parameter is particularly important in the evaluation of bread, as it serves as an overall indicator of consumer preference and satisfaction, which are essential for successful marketability (Tafti et al., 2013). In this study, sourdough bread made solely with wheat flour typically scored higher in overall acceptability than sourdough bread incorporating beetroot powder because of the inherent properties of sourdough, which enhance flavor complexity, texture, and moistness. The distinct sour notes and improved fermentation aspects contribute to a more balanced sensory profile that is generally favored over the potentially overpowering flavors or altered textures introduced by the beetroot powder.

Regarding the incorporation of beetroot powder in sourdough bread formulations, a 10% addition yielded the highest overall acceptability scores compared to lower (5%) or higher (15%) concentrations. This phenomenon can be attributed to the optimal balance of added flavor, color, and potential nutritional benefits, without overwhelming the bread's base character. Sensory assessments have indicated that at this concentration, the aroma, texture, and appearance are generally viewed as appealing, likely because this level enhances the visual appeal and flavor without introducing excessive earthiness or sweetness (Ani et al., 2022).

The 5% formulation may not provide sufficient distinctive qualities of beetroot for consumers to appreciate its presence, leading to lower acceptability scores. However, at 15%, beetroot integration may cause textural changes that detract from the chewy and satisfying qualities expected from sourdough bread (Babaoğlu et al., 2021). Overall, these findings underscore the importance of ingredient balance and sensory integration of various components in bread formulation to effectively cater to consumer preferences and optimize overall acceptability.

CONCLUSION

The incorporation of beetroot powder into sourdough bread significantly altered both the textural and sensory attributes, with effects proportional to the concentration used. Higher beetroot levels increased hardness, gumminess, chewiness, and stiffness due to the enhanced fiber content and altered gluten

network interactions. However, they also reduced springiness and, at excessive levels, negatively impacted the aroma, taste, and overall acceptability. Sensory analysis confirmed that 10% beetroot powder substitution achieved the best balance, offering improved color and distinctive flavor while maintaining a desirable sourdough texture and palatability. Conversely, 15% incorporation led to an undesirable dense crumb structure and reduced consumer liking. Therefore, for the development of functional bread products, moderate beetroot enrichment (approximately 10%) is recommended to optimize nutritional benefits without compromising quality or consumer preference.

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CLEAN LABEL APPROACH FOR FRESH GOAT MILK: ENHANCHING MICROBIAL SAFETY AND SHELF-LIFE USING HIGH HYDROSTATIC PRESSURE (HPP)

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ABSTRACT

The clean-label approach has gained global interest as consumers increasingly prefer minimally processed foods without synthetic additives. Concurrently, the demand for goat milk products is rising because of their nutritional and health benefits. This study investigated the application of high hydrostatic pressure (HHP) for controlling microbial growth and inactivating *Salmonella typhimurium* in fresh Saanen goat milk. Microbiological evaluation of HHP-treated milk samples included an *S. typhimurium* (ATCC 14028) challenge test and monitoring of total plate count, yeast and mold, coliforms, *Escherichia coli*, *Staphylococcus aureus*, lactic acid bacteria, and *Salmonella* during storage. Specifically, HHP was applied at 600 MPa for 6 min using food-grade spout-pouch packaging, based on prior optimization. HHP milk samples achieved complete inactivation of *S. typhimurium*, with reductions of 6 $\log_{10}$ CFU/mL at high spike levels and 3 $\log_{10}$ CFU/mL at low spike levels, demonstrating microbial decontamination kinetics. Shelf-life evaluation showed that HHP-treated milk retained acceptable microbiological, physicochemical, and sensory qualities for up to 75 d under chilled storage (2±7 °C), while fresh untreated milk spoiled by day 6, exceeding permissible microbial limits. Beyond 75 d, viscosity degradation was observed in HHP-treated milk. These findings confirm that optimized HHP treatment enhances microbial safety and extends shelf life, supporting its role in developing clean-label goat milk products.

Keywords: Clean label, fresh goat milk, high hydrostatic pressure, microbial safety, shelf life

INTRODUCTION

Milk is a key source of calcium, protein, and fat, supplying approximately 135 kcal per person daily. In Malaysia, the demand for milk is rising rapidly, and the National Dairy Industry Development Strategic Plan 2021–2025 targets 100% self-sufficiency by 2025 (Ministry of Agriculture and Food Industries, 2021). The National Agrofood Policy 2021–2030 (NAP 2.0) stated that milk (11.69% compound annual growth rate) is the agrifood product with the highest consumption rate, followed by mutton and poultry meat in Malaysia by 2025 (Ministry of Agriculture and Food Industries, 2021). Goat milk, valued for its nutritional content, is particularly susceptible to microbial growth due to its natural chemical composition, posing challenges in ensuring safe, high-quality products for consumers. Conventional thermal pasteurization effectively extends the shelf life of milk but can degrade milk nutrients and alter fat and protein structures, including reduced structural properties (e.g., fat globules and casein micelles) (Barraquio, 2014; Bogahawaththa et al., 2018). Studies have shown that thermal treatments cause 5–15% whey protein denaturation, 10–25% loss of water-soluble vitamins, and increased allergenicity of milk proteins (Bogahawaththa et al., 2018).

Alternatively, high hydrostatic pressure (HHP) offers a non-thermal technique that inactivates pathogens while preserving nutrients and fresh-like qualities (Evelyn and

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Silva, 2017). HHP is also called cold pasteurization because the process operates at ambient temperatures and destroys spoilage microorganisms through a pressure range of 300–600 MPa (Stratakos et al., 2019). Many studies have shown that HHP-treated milk can achieve an extended shelf life depending on pressure, duration, and storage temperature: 14–18 d at 200 MPa and 4 °C (Pereda et al., 2007), 18 d at 350 MPa and 4 °C (Mussa and Ramaswamy, 1997), and 28 days at 600 MPa (Alexandros et al., 2019). However, another study reported a shelf life of only 10 d for milk treated at 400 MPa for 15 min or 600 MPa for 3 min when stored at 10 °C, which was 18 days shorter than that at 4 °C (Rademacher and Kessler, 1997). Fresh milk commonly harbors pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Campylobacter* (Zobrist et al., 2005), which pose serious risks to human health. Alexandros et al. (2019) reported that HHP above 550 MPa for 3 min reduced *E. coli*, *Salmonella* spp., and *L. monocytogenes* to undetectable levels. Similarly, Yang et al. (2012) found that 300 MPa for 30 min effectively inactivated *Salmonella* spp., *E. coli*, *Shigella* spp., and *S. aureus*.

Nevertheless, most research on HHP-treated goat milk originates from temperate regions, with limited studies in tropical climates, such as Southeast Asia. Therefore, this study examined the effects of HHP on locally produced goat milk to develop a clean-label, microbiologically safe ready-to-drink (RTD) product with an extended shelf life, supporting organic and sustainable food production.

MATERIALS AND METHODS

Preparation of fresh goat milk upon pressurization study

Fresh Saanen goat milk (*Capra hircus*) was sourced from a local farm in Janda Baik, Pahang, Malaysia, and delivered on the same day of processing to the Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The milk was maintained at a chilled temperature (2±7 °C) during transportation to MARDI within 3 to 5 h for packaging into food-grade spout-pouch polyethylene terephthalate / aluminum / polyethylene (PET/AL/PE) with approximately 80 mL of sample per pouch. Packaged goat milk samples were processed using an HHP unit (Hiperbaric 55, Burgos, Spain) at the Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM). High pressure was applied to a cylindrical chamber using a pressure pump and hydraulic system, with water as the pressure medium. The processing conditions were set at 600 MPa for 6 min at 20 °C, as previously optimized using response surface methodology (RSM). In this study, HHP-treated milk was designated as 'HHP-treated milk,' while untreated samples ('fresh milk') served as controls. All samples were stored at low temperatures for further analysis.

Fresh goat milk samples were analyzed for pathogen challenge test on day 0 post-HHP and for shelf life evaluation in terms of microbiological, physicochemical, and sensory properties based on scheduled intervals. Fresh milk was analyzed up to day 6 at 3-d intervals for microbiological and physicochemical properties, whereas HHP milk was analyzed up to day 90 at 3-d intervals until day 6 and every 15 d thereafter. Sensory analysis was performed only on day 0 for fresh milk and monthly for HHP milk.

Pathogen Challenge Test

Salmonella analysis was performed on HHP milk and fresh milk for a challenge test using a method modified from the Bacteriological Analytical Manual (BAM, 2002). Pathogen challenge test was conducted using *Salmonella typhimurium* (ATCC 14028) at optimized HHP pressure of 600 MPa for 6 min. This specific test involved packaged milk that had been initially sterilized using an autoclave at 121 °C for 15 min. Then, *S. typhimurium* inoculum was inoculated into samples at concentrations of 10^3 CFU/mL as low spike and 10^6 CFU/mL as high spike at a ratio of 1 mL of culture in 80 mL of goat milk. Milk samples in spout packaging containing the inoculated *Salmonella* were filled and packed into nylon plastic as secondary packaging to prevent contamination in case of a leak during HHP treatment. Colonies showing typical reactions

on selective media (Xylose Lysine Deoxycholate and Xylose Lysine Tergitol-4: dark red with black centers; Rambach: bright red), as described by the manufacturer, were considered presumptive *Salmonella*. Well-isolated colonies were subjected to biochemical confirmation (Merck, Germany).

Shelf Life Evaluation

Microbiological Analysis

Microbial analysis of total plate count (TPC), yeast and mold (Y&M), lactic acid bacteria (LAB), coliform, *E. coli*, *S. aureus*, and *Salmonella* was conducted on HHP and fresh milk following the BAM (2002) and American Public Health Association (APHA, 2000) standard methods. The results were expressed as $\log_{10}$ CFU/mL, with plate counts based on 25–250 CFU/mL, except for 15–150 CFU/mL for Y&M. Microbiological tests were performed 1 h after HHP treatment, with all samples, including fresh milk controls, analyzed in triplicate.

Physicochemical Analysis

Determination of total soluble solids and pH

Total soluble solids (TSS) were measured using a pocket refractometer (Atago, Tokyo, Japan; 0–53 °Bx), and the sample pH was determined using a bench pH meter (FE20, Mettler Toledo, Switzerland) (AOAC, 2000).

Determination of viscosity

Viscosity was measured using a Tuning Fork Vibro Viscometer SV-10 with 45 mL samples placed in the provided containers (AOAC, 2000).

Color analysis (L*, a*, b* values)

The colors of HHP and fresh milk were measured using a Chroma Meter (CR-400/410, Konica Minolta, Japan) based on the CIELAB system (L*, a*, b*). The instrument was calibrated using a standard white plate. In this system, L* represents brightness from 0 (black) to 100 (white), a* indicates red (+) to green (–), and b* indicates yellow (+) to blue (–) (AOAC, 2000).

Sensory Analysis

A total of 40 untrained panelists (aged 21 to 58 years, healthy, and non-smokers) evaluated HHP-treated and fresh milk samples in the Food Sensory Laboratory, MARDI, under ambient temperature and fluorescent lighting. Using a seven-point hedonic scale (1 = strongly disliked to 7 = strongly liked), the panelists assessed the color, aroma, taste, viscosity, and overall acceptability. Samples with mean overall acceptability scores > 5.0 were considered acceptable (Granato et al., 2010).

RESULTS AND DISCUSSION

Pathogen Challenge Test

Table 1 summarizes the microbiological properties of fresh goat milk inoculated with *S. typhimurium* (ATCC 14028) and subsequently treated with HHP. In this study, control samples inoculated with low and high spike levels of *Salmonella* contained 3.50 and 6.74 $\log_{10}$ CFU/mL, respectively, chosen as worst-case scenario levels rather than realistic contamination levels, which are expected to be lower.

Table 1. Microbiological safety effectiveness of HHP-Treated on Goat Milk

Sample	Low spike ($\log_{10}$ CFU/mL)	High spike ($\log_{10}$ CFU/mL)
Fresh milk	3.50 ± 0.03	6.74 ± 0.02
HHP milk	Absent in 25 mL	Absent in 25 mL

Note: Data (mean±SD) were derived from three replicates. Fresh milk = fresh goat milk without HHP treatment followed by *S. typhimurium* inoculation (control). HHP milk = Sterilized fresh goat milk inoculated with *S. typhimurium* (low and high spike) followed by HHP treatment at 600 MPa for 6 min before storage at chilled (2 ±7 °C).

According to Food Standards Australia New Zealand (FSANZ, 2018) and the Centre for Food Safety Hong Kong (CFSHK, 2014), RTD products must be “absent in 25 mL” of RTD products for them to be deemed satisfactory (Table 2). HHP treatment at 600 MPa for 6 min reduced *Salmonella* to undetectable levels at both spike concentrations, with up to a 6 $\log_{10}$ CFU/mL reduction, consistent with Erkmen (2011) on *S. typhimurium* sensitivity to HHP. Similarly, Stratakos et al. (2019) reported that pressures of 400–600 MPa for 1–5 min achieved up to 5 log10 CFU/mL reduction in raw milk. From a microbiological perspective, the effectiveness

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of HHP in achieving food safety targets is often evaluated by a 5 log10 pathogen reduction, as required by the United States Food and Drug Administration (US-FDA, 2001) for compliance with Hazard Analysis Critical Control Point (HACCP) standards.

Shelf-Life Evaluation

Microbiological Analysis

HHP significantly reduced microbial counts in milk, with TPC, Y&M, and LAB decreasing by 4–5 $\log_{10}$ CFU/mL, coliforms by 3 $\log_{10}$ CFU/mL, and *S. aureus* by 2 $\log_{10}$ CFU/mL (Table 3), all within the international safety limits (Table 2). After 90 d of storage, HHP milk maintained satisfactory microbiological quality, in contrast to fresh milk, which showed rapid microbial growth and became unsafe within six days at 2 ±7 °C. TPC, a standard measure of microbial load in dairy products, is considered safe at ≥ 10^6 CFU/mL (FSANZ) or ≥ 10^7 CFU/mL (CFSHK, 2014). Fresh milk initially contained 10^4 CFU/mL (marginal) but exceeded 10^6 CFU/mL (unsatisfactory) after six days, whereas HHP milk was reduced to <1 CFU/mL and remained <10^2 CFU/mL for 90 d (Table 3).

Table 2. International Food Standards for microbiological safety limit

Analysis	Microbiological Safety Limit						
Bacterial Count (CFU/mL)	FSANZ				CFSHK		
	Satis-factory	Marginal	Unsatisfactory	Potentially Hazardous	Satis-factory	Marginal	Unsatis-factory
TPC	<10^4	10^4 - <10^6	≥10^6	-	<10^4	10^4 - <10^7	≥10^7
Yeast & Mold	-	-	-	-	-	-	-
LAB	-	-	-	-	-	-	-
Coliform	<10^2	10^2 - 10^4	>10^4	-	<10^2	10^2 - ≤10^4	>10^4
E. coli	<3	3 - <10^2	>10^2	-	<20	20 - ≤10^2	>10^2
S. aureus	<10^2	10^2 - <10^3	10^3 - ≤10^4	>10^4	<20	20 - ≤10^4	>10^4
Salmonella in 25 mL	Absent	-	Detected	Detected	Absent	-	Detected

Note: FSANZ, Compendium of Microbiological Criteria for Food (Food Standards Australia and New Zealand); CFSHK, Microbiological Guidelines for Food (Centre for Food Safety, Hong Kong). "-" = Not stated in the Food Standards for Microbiological Safety Limit.

Table 3. Microbiological safety effectiveness on HHP-Treated Goat Milk during storage period at chilled temperature (2-7 °C)

Analysis / Storage	TPC ($\log_{10}$ CFU/mL)	Yeast & Mold ($\log_{10}$ CFU/mL)	LAB ($\log_{10}$ CFU/mL)	Coliform ($\log_{10}$ CFU/mL)	*E. coli* ($\log_{10}$ CFU/mL)	*S. aureus* ($\log_{10}$ CFU/mL)	*Salmonella* in 25 mL
HHP-treated milk							
Day 0	n.d.	n.d.	0.50 ± 0.00	n.d.	n.d.	n.d.	Absent
Day 3	n.d.	n.d.	0.50 ± 0.00	n.d.	n.d.	n.d.	Absent
Day 6	n.d.	n.d.	0.50 ± 0.00	n.d.	n.d.	n.d.	Absent
Day 15	n.d.	n.d.	0.50 ± 0.00	n.d.	n.d.	n.d.	Absent
Day 30	n.d.	n.d.	0.50 ± 0.00	n.d.	n.d.	n.d.	Absent
Day 45	0.50 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	Absent
Day 60	0.50 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	Absent
Day 75	1.05 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	Absent
Day 90	1.22 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	Absent
Fresh milk							
Day 0	4.24 ± 0.06	4.24± 0.06	5.32 ± 0.05	3.43 ± 0.39	n.d.	2.74 ± 0.20	Absent
Day 3	5.77 ± 0.22	4.77 ± 0.37	5.68 ± 0.11	4.93 ± 0.03	0.50 ± 0.00	2.79 ± 0.23	Absent
Day 6	6.32 ± 0.22	4.84 ± 0.13	5.46 ± 0.06	5.12 ± 0.19	0.50 ± 0.00	3.28 ± 0.24	Absent
Day 7	Discontinued due to high microbial counts						

Note: Data (mean±SD)were derived from three replicates. HHP milk = Optimized HHP treatment at 600 MPa for 6 min before storage at chilled (2±7 °C), Fresh milk: Fresh goat milk without HHP treatment (control), Y = Yeast Count.

These findings align with previous studies reporting significant TPC reduction under HHP at 400-600 MPa for 3-10 min (Liepa et al., 2017; Razali et al., 2021; Tan et al., 2020; Lim et al., 2023). The TPC in HHP milk increased to 10^7 CFU/mL only after 28 d, compared with 14 d for pasteurized milk (Lim et al., 2023). Y&M can cause off-flavors in milk, limiting its shelf life and posing potential health risks (Liu et al., 2020). Fresh milk in this study contained 5.4×10^4 CFU/mL yeast, which was reduced to <10 CFU/mL after HHP treatment (Table 3). Similar findings were reported by Tan et al. (2020) and Lim et al. (2023), who found that most vegetative Y&M in cow and goat milk were inactivated within 5–7 min at 450–600 MPa and 10 min at 600 MPa, respectively. In this study, HHP maintained Y&M at undetectable levels for 90 days of storage, exceeding the 22- and 60-day durations reported by Tan et al. (2020) and Lim et al. (2023), respectively. LAB were reduced to <25 CFU/mL immediately after HHP (5 $\log_{10}$ CFU/mL reduction), although low levels persisted until day 30.

This is likely due to the thicker peptidoglycan cell walls of gram-positive bacteria, making LAB more pressure-resistant. The presence of LAB may be beneficial for probiotic properties in milk; however, LAB levels can also indicate sourness or the presence of pathogenic *Streptococci* (Barraquio, 2014).

Within this study, coliform (<1 CFU/mL) and *E. coli* (<1 CFU/mL) remained well below safety limits throughout 90 days of storage after HHP treatment (Table 3), far lower than the FSANZ and CFSHK thresholds of $>10^4$ CFU/mL for coliform and $>10^2$ CFU/mL for *E. coli* (Table 2). In contrast, fresh milk showed coliform counts exceeding marginal safety on day 0, which increased to unsatisfactory levels by day 6. This demonstrates the high efficacy of HHP treatment in ensuring milk safety, despite the potential microbial contamination during milking. Similar reductions have been reported: Stratakos et al. (2019) observed a 5.6–6.8 $\log_{10}$ CFU/mL reduction of *E. coli* at 600 MPa for 3–5 min; Liu et al. (2020) reported a 2.9 $\log_{10}$ CFU/mL reduction at 600 MPa for 5 min; and Tan et al. (2020) documented a 1.6 $\log_{10}$ CFU/mL reduction of coliform at 450–600 MPa for 5–7 min. Pathogenic *S. aureus* and *Salmonella* spp. remained undetected in HHP milk samples throughout 90 days of storage. In fresh milk, a high *S. aureus* count (3.28 $\log_{10}$ CFU/mL) was observed on day 6, exceeding the FSANZ limits and indicating spoilage. For safe consumption, *S. aureus* in RTD milk should be $<10^3$–10^4 CFU/mL, and *Salmonella* should be absent in 25 mL; both criteria were met in this study. The absence of *Salmonella* in fresh milk in this study may be attributed to good milking hygiene, local breeds, and the cool climate of Janda Baik, Malaysia. Notably, Chye et al. (2004) reported *Salmonella* contamination of 1.4% in raw cow milk samples in Malaysia. These results are consistent with those of Tan et al. (2020), who found that HHP (600 MPa for 3 min) reduced *S. aureus* and *Salmonella* more effectively than pasteurization.

Effects of HHP on Physicochemical Properties

The physicochemical properties of HHP and fresh milk are shown in Table 4. The pH of HHP milk remained stable (6.44–6.69) over 90 d, whereas of that fresh milk ranged from 6.06 to 6.46 over 6 d. These results indicate minimal pH changes, consistent with previous studies reporting no significant pH differences in HHP-treated goat (Mustapa Kamal et al., 2021; Park et al., 2007) and cow milk (Tan et al., 2020). However, some studies observed pH increases after HHP treatment; Tan et al. (2020) (450–600 MPa for 7 min) in goat milk, Liepa et al. (2017) (500–600 MPa for 15 min) in cow milk, and Zobristal et al. (2005) (100–600 MPa for 30 min) in cow milk. Such variations may result from HHP-induced changes in the mineral distribution, including calcium, phosphate, and other ions (Zobristal et al., 2005). Goat milk naturally has a lower pH than cow milk because of its higher acidity (Mahmood and Usman, 2010).

Overall, our study showed that HHP treatment had minimal effect on pH, °Bx, and color of milk over 90 d of storage compared to controls (Table 4). However, the viscosity increased on day 75 (4.94 ± 0.03 mPas) and day 90 (6.57 ± 0.02 mPas). This gradual

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increase likely reflects pressure-induced modifications of milk proteins, leading to their aggregation during storage. HHP disrupts casein micelles and alters whey protein conformations, promoting protein–protein interactions and the formation of larger aggregates or gel-like networks, which increase viscosity and alter rheological behavior. Similar trends have been observed in bovine and small ruminant milks, which are attributed to casein micelle fragmentation, whey protein denaturation, and calcium redistribution, followed by slow reassociation and disruption over time (Serna-Hernandez et al., 2021).

Table 4. The physicochemical profile of HHP-treated goat milk during storage period at chilled temperature

Length of storage (d)	pH	Total soluble solids (°Bx)	Viscosity (mPas)	Color		
				L*	a*	b*
HHP milk						
0	6.61 ± 0.02	11.00 ± 0.00	2.04 ± 0.07	61.07 ± 0.03	1.02 ± 0.01	0.35 ± 0.05
3	6.53 ± 0.04	10.00 ± 0.00	2.18 ± 0.08	67.59 ± 0.01	2.70 ± 0.01	1.15 ± 0.01
6	6.55 ± 0.05	10.67 ± 0.12	2.69 ± 0.02	68.24 ± 0.00	2.60 ± 0.01	4.69 ± 0.01
15	6.44 ± 0.10	10.73 ± 0.12	4.18 ± 0.08	68.63 ± 0.06	2.61 ± 0.01	4.51 ± 0.01
30	6.69 ± 0.00	10.77 ± 0.06	4.25 ± 0.06	66.55 ± 0.02	2.08 ± 0.02	2.98 ± 0.01
45	6.58 ± 0.01	10.00 ± 0.00	4.44 ± 0.13	68.50 ± 0.36	2.56 ± 0.02	3.89 ± 0.02
60	6.69 ± 0.00	10.73 ± 0.12	4.21 ± 0.04	66.94 ± 0.03	2.16 ± 0.02	2.98 ± 0.01
75	6.52 ± 0.01	10.60 ± 0.00	4.94 ± 0.03	68.80 ± 0.10	2.49 ± 0.09	3.88 ± 0.06
90	6.52 ± 0.02	10.67 ± 0.06	6.57 ± 0.02	68.03 ± 0.25	2.49 ± 0.09	3.82 ± 0.01
Fresh milk						
0	6.46 ± 0.02	11.00 ± 0.00	1.84 ± 0.01	87.99 ± 0.01	-4.51 ± 0.11	11.57 ± 0.02
3	6.06 ± 0.01	9.50 ± 0.20	1.83 ± 0.06	79.94 ± 0.01	-3.05 ± 0.01	5.48 ± 0.01
6	6.45 ± 0.01	9.97 ± 0.15	3.81 ± 0.07	74.43 ± 0.01	-2.66 ± 0.01	4.14 ± 0.00
7	Discontinued due to high microbial counts					

Note: Data (mean±SD) were derived from three replicates. HHP milk = Optimized HHP treatment at 600 MPa for 6 min before storage at chilled (2±7 °C), Fresh milk = Fresh goat milk without HHP treatment (control).

Effects of HHP on Sensory Properties

Table 5 shows the sensory scores of HHP milk and fresh milk, indicating only slight differences in color, aroma, taste, viscosity, and overall acceptability, demonstrating the effectiveness of the HHP treatment. During the 90-d storage of HHP milk, viscosity decreased from 5.8±1.2 at month 2 to 4.5±0.8 at month 3, with only minor declines in overall acceptability (4.8), indicating gradual quality changes, likely due to protein aggregation and mild fat oxidation. In contrast, fresh milk initially scored high (overall acceptability 6.1 on day 0) but was rejected after one month due to microbial spoilage, consistent with its physicochemical instability.

Table 5. Sensory Analysis of HHP-Treated Goat Milk Throughout Storage Period at Chilled Temperature

Sensory characteristics	HHP-treated milk storage (month)				Fresh milk storage (month)	
	0	1	2	3	0	1
Color	6.2 ± 0.8	6.4 ± 0.7	6.4 ± 0.8	6.0 ± 0.6	6.2 ± 0.9	Discontinued to high microbial counts
Aroma	5.7 ± 1.0	5.9 ± 0.9	5.8 ± 1.0	5.2 ± 0.7	5.8 ± 0.9	
Taste	5.8 ± 0.7	5.9 ± 1.1	5.7 ± 1.0	5.1 ± 0.5	5.7 ± 0.9	
Viscosity	5.7 ± 0.9	6.1 ± 0.9	5.8 ± 1.2	4.5 ± 0.8	5.8 ± 0.8	
Overall acceptability	6.0 ± 0.6	6.1 ± 0.9	6.0 ± 0.8	4.8 ± 0.9	6.1 ± 0.6	

Note: HHP milk = Optimized HHP treatment at 600 MPa for 6 min prior to storage at chilled (2 °C ± 7 °C), Fresh milk = Control (Fresh goat milk without HHP treatment). All results were averaged over three replicates.

Previous studies have shown that goat milk experiences significant protein oxidation and aggregation during cold storage, as reflected by increased malondialdehyde levels, turbidity, and particle size, which correlate with sensory decline. HHP treatment slows but does not entirely prevent lipid oxidation and free fatty acid accumulation, especially at higher pressures or longer durations (Zhu et al., 2025). Sensory analysis at day 60 still indicated acceptable overall acceptability (>6.0) with satisfactory microbial results; however, viscosity quality gradually declined on day 90. Considering the overall sensory, physicochemical, and microbiological properties, HHP milk remained satisfactory for up to 75 d under chilled conditions. Therefore, the optimized HHP parameters within this study effectively extended the shelf life of milk up to 75 d, compared to fresh milk, which exceeded microbial limits and spoiled by day 6. These findings are supported by other studies that demonstrated quality preservation in clean-label products, such as fresh milk (Roobab et al., 2023; Lim et al., 2023).

CONCLUSION

HHP treatment at 600 MPa for 6 min effectively inhibited *S. typhimurium*, achieving a maximum reduction of 6 $\log_{10}$ CFU/mL in fresh goat milk and ensuring a robust food safety margin. The optimized process also extended the shelf life of HHP-treated milk to 75 d at chilled (2±7 °C) while maintaining satisfactory microbiological, physicochemical, and sensory quality. In contrast, untreated milk spoiled by day 6, with microbial counts exceeding permissible limits. Overall, HHP is a promising technique for producing clean-label, minimally processed RTD fresh goat milk that complies with international standards for food safety.

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THE EFFECT OF CARBOXYMETHYL CELLULOSE AND GLYCEROL ADDITION ON THE BIODEGRADABILITY OF CASSAVA STARCH-BASED BIOFILMS

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ABSTRACT

The growing concern over plastic pollution has accelerated the development of biodegradable packaging materials derived from natural polymers. Cassava starch has been widely studied as a potential raw material because of its abundance and biodegradability. However, its properties are often modified with additives such as carboxymethyl cellulose (CMC) and glycerol to improve film performance, which may influence its biodegradation. This study aimed to investigate the effects of different concentrations of CMC and glycerol on the biodegradability of cassava starch-based biofilms. Biofilms were prepared using three levels of cassava starch (5, 10, and 15 g), three concentrations of CMC (1, 2, and 3%), and three levels of glycerol (6, 9, and 12 mL). The biodegradability test was performed using the soil burial method for seven days, with weight loss as the primary parameter. The results showed that all biofilm samples achieved more than 60% degradation within seven days, thus meeting the Indonesian National Standard (SNI) for biodegradability. At low starch concentrations (5 g), the addition of higher CMC and glycerol levels reduced biodegradability due to matrix compaction and reduced porosity. At 10 g starch, biodegradability was more stable, with several formulations reaching 100% degradation by day seven. The highest starch concentration (15 g) yielded the best results, with nearly all formulations reaching complete biodegradation, supported by sufficient substrate availability and synergistic interactions between CMC and glycerol. Overall, the findings indicate that starch concentration plays a dominant role, whereas excessive CMC and glycerol can slow degradation by limiting microbial accessibility.

Keywords: Biodegradability, Biofilm, Carboxymethyl Cellulose, Cassava Starch, Glycerol

INTRODUCTION

Conventional plastics pose a major environmental challenge because of their resistance to natural degradation, leading to long-term accumulation and pollution (Jiang et al., 2020). Indonesia ranks among the world's largest contributors to plastic waste, with significant amounts entering both terrestrial and marine ecosystems. This underscores the urgent need for sustainable alternatives to conventional plastic films. One potential solution lies in biodegradable plastics, particularly biodegradable films, which can be decomposed by microorganisms into environmentally benign products (George et al., 2021).

Among the various forms of biodegradable plastics, biodegradable films have attracted particular attention owing to their wide application in packaging, especially as replacements for conventional plastic films. Cassava starch is considered a highly promising biodegradable film due to its abundance, affordability, and high starch content, which can reach up to 90% (Cheng et al., 2021). Moreover, Indonesia, as one of the largest cassava-producing countries, has significant potential to utilize this resource for the development of biopolymers. Nevertheless, starch-based films generally suffer from limitations, such as low mechanical strength and poor flexibility,

necessitating the incorporation of reinforcing and plasticizing agents.

In starch-based biodegradable films, the incorporation of a plasticizer is essential to overcome the inherent brittleness caused by strong intermolecular hydrogen bonding within the polymer matrix (Hidayat et al., 2023). Glycerol is one of the most widely used plasticizers in biodegradable film systems because of its low molecular weight, high compatibility with hydrophilic biopolymers, and multiple hydroxyl groups that enable effective interaction with starch and cellulose derivatives. By partially replacing polymer–polymer hydrogen bonds with polymer–glycerol interactions, glycerol increases chain mobility, resulting in improved flexibility, elongation, and film integrity (Giubertoni et al., 2023). However, glycerol is hygroscopic, and excessive concentrations can increase water uptake and reduce the water resistance of the films (Affanti et al., 2024). Therefore, optimizing the glycerol content is critical for achieving a balance between mechanical flexibility and barrier performance in starch–CMC biodegradable films.

Carboxymethyl cellulose (CMC) has been widely applied as a reinforcing filler to improve the mechanical and barrier properties of starch-based films (Cui et al., 2021), whereas glycerol is commonly used as a plasticizer to enhance flexibility and reduce brittleness (Fahrullah and Ervandi, 2022). However, the inclusion of these additives may also influence the biodegradability of the films, which is a critical parameter that determines their environmental compatibility.

This study aimed to analyze the effects of varying concentrations of cassava starch, CMC, and glycerol on the biodegradability of cassava starch-based biofilms. The results were evaluated with reference to the Japanese Industrial Standards (JIS) and Indonesian National Standards (SNI), contributing to the development of biofilm formulations that balance functional performance with environmental sustainability.

MATERIALS AND METHODS

Materials

Cassava starch was extracted locally and used as the primary raw material for the study. Carboxymethyl cellulose (CMC) and glycerol were used as additives to modify the film properties. Distilled water was used to prepare the film-forming solutions, and garden soil was used as the medium for the soil burial biodegradability test.

Experimental Design

A factorial experimental design was applied with three levels of cassava starch concentration (5, 10, and 15 g), three levels of CMC concentration (1, 2, and 3% w/w based on starch), and three levels of glycerol volume (6, 9, and 12 mL). This resulted in 27 unique treatment combinations being generated.

Biofilm Preparation

Biofilm-forming solutions were prepared by dissolving a specified amount of cassava starch in distilled water and heating the mixture to 75 °C until gelatinization. Subsequently, CMC and glycerol were added in accordance with the experimental design and stirred until homogeneous. The mixtures were then cast onto molds and dried in an oven at 60°C for 24 h to obtain thin biofilms. This method was used by Brion-Espinoza et al. (2021) with minor modifications to the original method.

Biodegradability Test

A biodegradation test was performed using soil microorganisms to assist in the degradation process, which is known as the soil burial test (Natalia et al., 2019). The biodegradability of the biofilm samples was evaluated using the soil-burial method. Film specimens measuring 3 × 3 cm were buried at a depth of 10 cm in garden soil, with soil moisture maintained at approximately 50–60% of water-holding capacity and temperature controlled at 25–30 °C to ensure consistent microbial activity. The samples were retrieved daily over a seven-day period, carefully cleaned of soil residues, dried, and weighed to monitor their weight losses over time. The percentage of biodegradation was determined by comparing the initial dry weight of each sample before burial with the dry weight after retrieval, expressing the loss as a percentage. This method provides a quantitative measure of the biofilm degradation rate in a natural soil environment.

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Biodegradability was measured using the following equation:

$$\text{Biodegradability (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

This formula was used to calculate the biodegradability of the samples based on weight changes during the degradation process (Acharjee et al., 2023).

RESULTS AND DISCUSSION

This biodegradability test was conducted to determine the ability of soil microorganisms to degrade plastic films. The test was carried out using The Soil Burial Test method, in which the samples were buried in soil and their weights were observed before and after burial for seven days (Kuswytasari et al., 2019). All cassava starch–based biofilm samples fulfilled the Indonesian National Standard (SNI) requirement of >60% degradation within seven days, with several formulations achieving complete degradation in less than a week.

Starch concentration was the dominant factor influencing biodegradability: higher starch levels (15 g) provided an abundant substrate for microorganisms, resulting in the fastest and most uniform degradation, whereas low starch levels (5 g) made the films more sensitive to the effects of CMC and glycerol. At 5 g starch, excessive CMC and glycerol reduced porosity and slowed degradation, whereas at 10 g starch, biodegradability became more stable, and several formulations achieved full degradation by day 7 (Fig 1.).

At 15 g starch, nearly all formulations reached 100% degradation, with the optimal formula of 15 g starch, 1% CMC, and 6 mL glycerol showing complete degradation by day five. Overall, starch content primarily controlled degradation, whereas appropriate levels of CMC and glycerol enhanced flexibility, porosity, and microbial accessibility. These findings highlight the importance of balancing starch concentration with additive ratios to produce biofilms that are both mechanically stable and environmentally degradable, underscoring their potential for sustainable food-packaging applications.

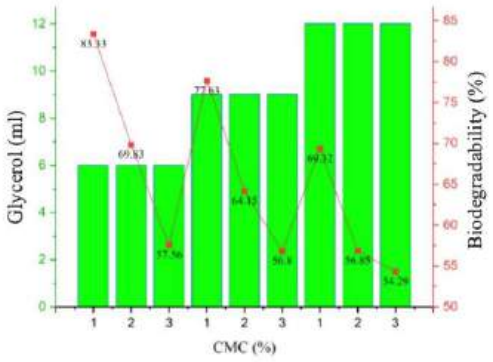

Figure 1. Graph of the effect of the CMC and glycerol combination on the biodegradability test rate with 5 g cassava starch concentration.

In the cassava starch-based films containing 5 g of starch, the addition of CMC and glycerol significantly influenced biodegradability. CMC reinforced the film structure, which tended to slow down the degradation process, particularly at higher concentrations. Meanwhile, glycerol, as a plasticizer, increased the flexibility of the film; however, excessive glycerol led to a more homogeneous matrix with reduced porosity, making it more difficult for microorganisms to access the starch substrate.

At low starch concentrations, the main components, amylose and amylopectin, were relatively accessible; therefore, the effects of CMC and glycerol became more dominant in determining the biodegradation rate. The optimal combination of 1% CMC and 6 mL glycerol showed the highest biodegradability, as the film matrix remained sufficiently porous and flexible to facilitate the microbial activity. Conversely, formulations with higher concentrations, such as 3% CMC and 12 mL of glycerol, exhibited lower biodegradability (down to 83.09%) owing to a denser and more complex polymer network. Overall, the combination of CMC and glycerol at low starch levels enhanced film porosity, solubility, and flexibility, collectively accelerating microbial access and improving the biodegradation rate.

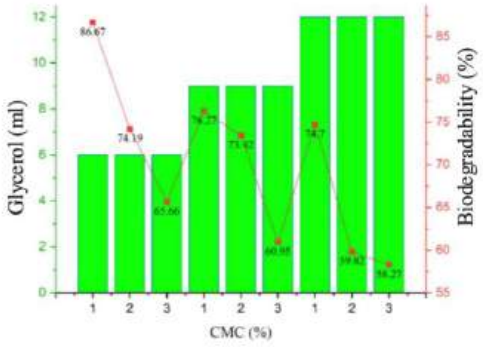

Figure 2. Graph of the Effect of CMC and Glycerol Combination on Biodegradability Rate with 10 g Cassava Starch Concentration

Figure 2 shows that for 10 g of cassava starch, the combination of CMC and glycerol affects biodegradability in a manner similar to that of 5 g of starch, but with notable differences owing to the increased film matrix density. The highest biodegradability reached 100% on day 7 for certain combinations, such as 1% CMC with 6 mL of glycerol. However, at higher concentrations (e.g., 3% CMC and 12 mL glycerol), biodegradability increased to 87.07%, not due to a higher starch content, but due to the enhanced accessibility of starch chains to microbial and enzymatic attacks (Kuswytasari et al., 2019).

Although CMC forms a denser polymer network through hydrogen bonding with starch, the high glycerol content increases matrix plasticization, water uptake, and free volume, facilitating microbial penetration and accelerating the biodegradation process. The difference arises from the matrix structure, porosity, and plasticization rather than the starch quantity (Ramakrishnan et al., 2024). CMC acts as a thickener and matrix-strengthening agent, forming a denser polymer network through hydrogen bonding with starch, which limits microbial access and slows degradation.

At high CMC concentrations, the matrix becomes rigid, reducing biodegradability (Fig. 3). Similarly, glycerol enhances film flexibility but reduces porosity, creating a more homogeneous matrix that is harder to penetrate by microorganisms. Compared to 5 g starch, the 10 g starch films had a denser and more organized matrix, which balanced mechanical strength and flexibility. This allows for higher and more stable biodegradability, especially with optimal combinations of CMC and glycerol (e.g., 1% CMC with 6–9 mL glycerol), as starch molecules are more available and accessible to microorganisms. However, high additive concentrations can negatively affect biodegradability by increasing matrix density and limiting microbial access.

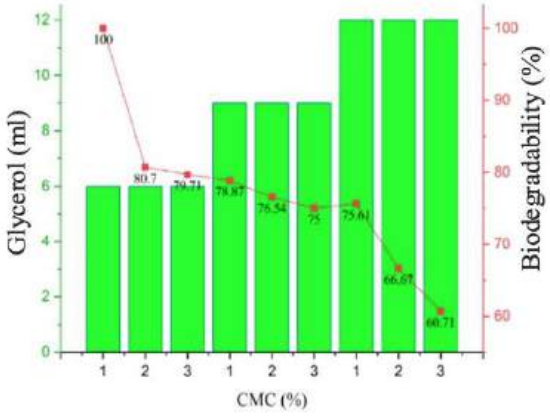

Figure 3. Effect of CMC and glycerol on biodegradability of 15 g cassava starch films

The graph in Figure 3 shows that films containing 15 g of cassava starch exhibited very high biodegradability, with most formulations reaching 100% degradation by day 7. Higher starch concentrations provide abundant substrates in the form of amylose and amylopectin, facilitating microbial activity and accelerating degradation (Munfarida and Hidayat, 2023). Despite the denser matrix compared to 10 g starch films, glycerol contributed to flexibility and sufficient porosity, allowing microorganisms to access the starch effectively. CMC strengthens the polymer network while maintaining hydrophilicity, improving water absorption, and softening the film structure, which enhances microbial access. Glycerol, a plasticizer, increases matrix flexibility, creates intermolecular spaces, and attracts moisture, further supporting the biodegradation process (Dewi et al., 2021). The synergistic combination of CMC and glycerol is suggested to enhance matrix flexibility and permeability, which can facilitate water diffusion and microbial access even at high starch concentrations (Munfarida and Hidayat, 2023), thereby promoting efficient

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biodegradation. Compared to the 5 g and 10 g starch films, the 15 g starch films showed the highest and most stable biodegradability owing to the greater substrate availability and optimal matrix properties. All cassava starch-based films met the Indonesian National Standard (SNI) for biodegradability, requiring at least 60% degradation within a week, with some formulations achieving complete degradation in less than seven days. The 15 g starch film with 6 mL of glycerol performed best, reaching 100% degradation in less than a week, demonstrating its eco-friendly potential and suitability for food packaging applications.

The three graphs (Fig 1–3) collectively illustrate the influence of cassava starch concentration on the biodegradability of the films in combination with CMC and glycerol. For films containing 5 g starch, biodegradability was moderately high but variable. Low starch concentrations created a loosely organized matrix, rendering the films highly sensitive to the effects of CMC and glycerol. At this level, CMC reinforced the film structure, whereas glycerol increased the flexibility. However, excessive concentrations of either additive reduced biodegradability by creating denser or overly homogeneous matrices, thereby limiting microbial access to the starch substrate. The optimal combination (1% CMC and 6 mL glycerol) resulted in the highest biodegradability, suggesting enhanced matrix permeability and chain mobility, which facilitated microbial activity. In films with 10 g starch, biodegradability became more stable and was generally higher than that in the 5 g starch films. The increased starch content provides a larger substrate pool of amylose and amylopectin, allowing microorganisms to degrade the polymer network more effectively (Tapia-Blácido et al., 2022). Although high concentrations of CMC and glycerol slightly reduced biodegradability by stiffening the matrix or reducing porosity, many formulations, including 1% CMC with 6–9 mL of glycerol, reached complete degradation (100%) by day 7. The denser but more organized matrix at this starch concentration balanced structural strength with flexibility, supporting more consistent microbial access across the film. The biodegradability of the films containing 15 g starch was the highest and most uniform among all concentrations. Almost all the formulations reached 100% degradation by day 7. The abundant starch substrate enhances microbial activity, whereas the synergistic effects of CMC and glycerol maintain a flexible and porous matrix despite the higher polymer density (Bergel et al., 2020). CMC reinforced the network and improved water absorption, facilitating matrix softening, while glycerol further increased flexibility and hygroscopicity, promoting microbial penetration. Notably, the formulation with 15 g starch and 6 mL glycerol exhibited the fastest and most complete degradation, highlighting the optimal balance between the polymer concentration and additive effects.

Overall, the comparison of the three starch levels demonstrated that increasing the starch concentration enhanced biodegradability by providing more substrate for microorganisms and stabilizing the matrix. At low starch concentrations, additive effects dominate, occasionally hindering biodegradability. At higher starch levels, the matrix was robust yet accessible, allowing CMC and glycerol to synergistically improve the film properties without compromising degradation. These results confirm that the proper adjustment of starch concentration and additive ratios is crucial for achieving films that are both mechanically stable and environmentally degradable.

CONCLUSION

All cassava starch–based biofilms met the SNI requirement of >60% degradation within seven days, with several formulations achieving complete degradation in less than a week. Starch concentration was the key determinant of biodegradability, as higher starch levels (15 g) supplied more substrate for microbial activity, resulting in faster and more uniform degradation, whereas lower starch levels (5 g) made the films highly influenced by CMC and glycerol content. Conversely, at low starch concentrations, the effect becomes more pronounced because of the inherently less dense polymer matrix. A low CMC content (1%) provides sufficient network stabilization without excessively

limiting microbial access. Excessive additives tend to create denser matrices that limit microbial access, whereas optimal combinations improve porosity, flexibility, and water absorption to support degradation. At 10 g starch, biodegradability became more stable, and several formulations reached 100% by day 7, whereas at 15 g starch, nearly all formulations achieved full degradation, with the best result (15 g starch, 1% CMC, and 6 mL glycerol) degrading completely by day 5. These findings confirm that the proper adjustment of starch concentration with CMC and glycerol is essential to balance mechanical stability and environmental degradability, making cassava starch biofilms a promising material for sustainable packaging applications.

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QUALITY EVALUATION OF SALAK PEEL TEA UNDER VARIOUS DRYING TEMPERATURES AND DURATIONS

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ABSTRACT

Salak peel, commonly discarded as agricultural waste, contains bioactive compounds that can be developed into functional food products, such as herbal tea. The quality of this tea is significantly influenced by the drying process, particularly in terms of temperature and drying duration. This study investigated the effects of different drying temperatures and durations on the sensory and nutritional characteristics of salak peel tea. Drying was carried out at three temperature levels (60, 70, and 80°C) combined with six drying durations (2, 4, 6, 8, 10, and 12 h). Sensory attributes, including color, aroma, and taste, were evaluated, and the data were analyzed using factorial ANOVA, followed by Duncan's multiple range test at a 5% significance level. The findings revealed that temperature and drying time significantly affected the sensory quality of the salak peel tea. The optimum conditions were observed at 70°C for 4 h, producing scores of 3.8, 3.76, and 3.5 for color, aroma, and taste, respectively. Proximate analysis of the best treatment indicated a moisture content of 3.85%, ash of 5.77%, protein of 0.55%, fat of 0.06%, and carbohydrate of 89.77%. These results suggest that drying at 70°C for 4 h is the most suitable treatment for producing salak peel tea with desirable sensory characteristics and proximate composition.

Keywords: Salak peel; drying method; organoleptic

INTRODUCTION

Indonesia has a high potential for local fruit production. Salak (*Salacca zalacca*) is an indigenous fruit of Indonesia and a major horticultural commodity. Salak comes from the palm family, where the fruit grows in clusters at the base of the tree, covered with reddish-brown scales that resemble snakes; hence, the name snake fruit (Saleh et al., 2018a). The fruit is egg-shaped, with white, firm flesh and a sweet, slightly sour, and astringent taste (Suica-Bunghez et al., 2016). This fruit is underutilized but is rich in carbohydrates, dietary fiber, and antioxidants, which vary slightly depending on the variety (Mazumdar et al., 2019). The fertile soil of Kalimantan is ideal for salak cultivation, resulting in highly productive harvests. This is especially true in Balikpapan, East Kalimantan, which serves as a major hub for the cultivation of fruits.

According to 2025 data from BPS-Statistics Balikpapan City, salak production reached 109,641 quintals, driven primarily by the North Balikpapan District, salak production reached 109,641 quintals, with the largest contributor being the North Balikpapan District. This production has increased significantly compared to the previous year, when it was only 47,902 quintals (BPS, 2025). The greater the volume of salak fruit harvested, the greater the quantity of salak peels produced (Kusdarini et al., 2025). As long as the consumer only focuses on using the fruit flesh, the peel is discarded directly into the environment as waste. The whole salak fruit has an edible portion of 56-65%, while 35-44% is considered waste, with 10-14% being the peel (Surbakti and Barus, 2022). Instead of allowing salak peels to become agricultural waste, they can be transformed into valuable products.

By-products, such as fruit peels, contain high levels of bioactive compounds. Research on salak (*Salacca zalacca*) peel has mainly focused on phytochemical identification rather than processing optimization. Studies

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related to the drying of salak peel remain limited, even though drying is a crucial step in the production of herbal tea and greatly influences the product quality (Kowalska et al., 2017). To date, there is no established agreement on the most appropriate drying method or optimal conditions, such as temperature and drying duration, to effectively retain phenolic compounds and antioxidant activity in salak peel tea (Girsang et al., 2021). Moreover, the effects of drying parameters on the functional stability and sensory characteristics of salak peel tea have not been systematically evaluated, particularly in relation to its radical scavenging potential and health-related properties (Tan et al., 2020). Research on salak (*Salacca zalacca*) peel has primarily focused on phytochemical identification rather than processing optimization. Studies related to the drying of salak peel remain limited, even though drying is a crucial step in the production of herbal tea and greatly influences the product quality. Furthermore, the antioxidant activity of the peel was recorded at 229.27 ± 6.35 µg/mL, highlighting its potential as a functional ingredient (Čepková et al., 2021). This gap highlights the need for targeted drying optimization studies to support the development of salak peel as a functional tea product and enhance its utilization as an agricultural by-product.

Complementary evidence from herbal tea studies, such as that reported by Wickramarachchi et al. (2017), has demonstrated that drying temperature and duration significantly influence the nutritional composition and sensory quality of herbal teas. The longer the duration and the higher the temperature, the greater the effect on the characteristics of the resulting tea (Smith et al., 2024). This study investigated the effects of different drying temperatures and durations on the sensory and nutritional characteristics of salak peel tea. The quality of the salak peel tea was evaluated using organoleptic tests based on color, aroma, and taste. Proximate analysis was performed to evaluate the nutritional composition and overall quality of the tea. Collectively, these findings support the rationale of the present study, indicating that optimization of drying temperature and time is essential for enhancing visual quality.

MATERIALS AND METHOD

Materials

Ripe fruits of *Salacca zalacca* were sourced from local farmers at KM 21, Balikpapan City, East Kalimantan, Indonesia. Only fruits with intact peels and free from visible defects were selected. The outer skin was separated manually and used as raw material. Distilled water was used for cleaning, and food-grade filter paper was used for tea bag preparation. Analytical grade chemicals were used for proximate analysis.

Experimental Design and Data Analysis

A factorial experiment was conducted to evaluate the effects of drying temperature and time on the salak peel tea. The treatments consisted of three temperature levels (60, 70, and 80 °C) combined with six drying durations (2, 4, 6, 8, 10, and 12 h), each performed in triplicate. Sensory evaluation, comprising color, aroma, and taste, was conducted using a five-point hedonic scale, and nutritional attributes were determined according to standard procedures. Data were analyzed using two-way ANOVA, and mean separation was performed using Duncan's test at a 5% significance level. The treatment combination with the highest sensory acceptance was further analyzed for its proximate composition following AOAC standard methods.

Methods

Fresh salak (*Salacca zalacca*) fruits were sourced directly from local farmers in Balikpapan, East Kalimantan. The peels were manually removed, thoroughly rinsed with running water, and allowed to drain at ambient temperature for approximately 10 min before weighing. Subsequently, the peels were cut into uniform pieces with a thickness of 2–3 mm and dimensions of approximately 2 × 3 cm to ensure consistent drying behavior.

The prepared peel samples were placed evenly in a single layer on stainless steel drying trays, with a loading of approximately 200 g per tray and a spacing of 2–3 mm between individual pieces to facilitate uniform air distribution. Drying was performed in an electric cabinet-type food dehydrator equipped with forced convection. The samples were dried at 60, 70, and 80 °C

for drying durations of 2, 4, 6, 8, 10, and 12 h. Each treatment was performed in three independent replicates, following the experimental design (Rachmadani et al., 2025). This temperature range was selected based on prior hot-air drying studies on salak fruit and peel products, which demonstrated that drying temperature plays a critical role in moisture reduction, drying kinetics, and product quality. Upon completion of drying, the samples were cooled to room temperature for 30 min and then stored in airtight, food-grade containers until further analysis.

Analysis

Hedonic Evaluation

A sensory evaluation was conducted to assess the consumer acceptance of salak peel tea in terms of color, aroma, and taste. Twenty-five untrained panelists, recruited from undergraduate students at Institut Teknologi Kalimantan, participated in the test. **S**ensory evaluation was conducted in a quiet, well-ventilated room under controlled neutral lighting, with panelists isolated in individual booths to prevent interaction. Tea samples were prepared under standardized conditions by brewing 2 g of dried salak peel powder in 200 mL of hot water (95 °C for 5 min) and served in coded cups labeled with randomized three-digit numbers to ensure blind testing of the samples. Each panelist independently evaluated all samples and scored them using a five-point hedonic scale ranging from 1 (dislike very much) to 5 (like very much) (Djaafar et al., 2024). The scores were recorded for each attribute, and mean values were calculated to determine the most preferred sample, which was selected for proximate analysis.

Proximate Analysis

A procedure to quantify the major nutritional fractions, including moisture, ash, crude protein, crude fat, and carbohydrates (by difference), was conducted according to the Official Methods of Analysis of AOAC International (AOAC, 2006). Moisture and ash were determined gravimetrically, crude protein was measured using the Kjeldahl nitrogen method, crude fat was obtained by Soxhlet extraction with a non-polar solvent, and carbohydrate was calculated as the remaining portion after subtracting the other components from 100%.

Moisture content

Moisture content was determined gravimetrically by drying approximately 5 g of the ground sample in a hot-air oven at 105 °C until a constant weight was achieved. The moisture content was calculated based on the weight loss relative to the initial mass.

Ash content

Ash content was determined using the gravimetric method. Approximately 2–3 g of the sample was placed in a pre-weighed crucible, charred, and then incinerated in a muffle furnace at 550 °C until a light-colored ash was obtained. The remaining residue was weighed, and the ash percentage was calculated based on the original sample mass.

Crude protein

Protein content was analyzed using the Kjeldahl method. The sample was digested in concentrated sulfuric acid with a catalyst until clear, followed by the distillation and titration of the released ammonia. The nitrogen content was determined and converted to crude protein using a 6.25 factor.

Crude fat

Fat content was determined using Soxhlet extraction. A known weight of dried sample was wrapped in filter paper and placed in an extraction thimble, and extracted with petroleum ether for 6–8 h. After solvent removal, the extract was dried and weighed, and the percentage of fat was calculated.

Carbohydrate (by difference)

The carbohydrate content was estimated by difference using the following formula:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Fat})$$

RESULTS AND DISCUSSION

Salak Peel Tea

The product obtained in this study was salak peel tea powder, a functional herbal tea derived from the dried peel of salak fruit (*Salacca zalacca*). This powder is produced by washing, slicing, and drying the peels, followed by milling them into a fine powder. The resulting product retains desirable sensory characteristics, such as color, aroma, and taste, as well as nutritional components.

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The effects of different drying conditions on the quality of the best salak peel tea powder are illustrated in Figure 1.

(a) (b)

Figure 1. Salak peel tea powder with treatments (a) 70°C for 4 hours and (b) 60°C for 12 hours

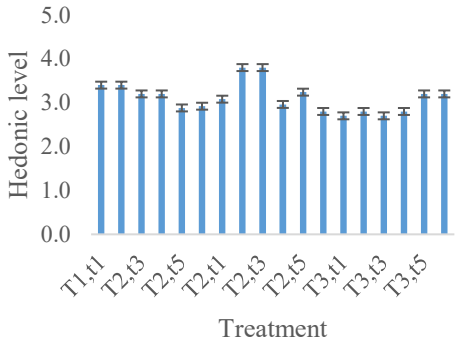
The following figure illustrates the salak peel tea powder produced under optimal treatment, representing the best combination of drying temperature and time. Among the treatments, the sample dried at 70 °C for 4 h was selected as the optimal condition. Although there was no significant difference in sensory scores compared to the sample dried at 60 °C for 12 h, the shorter drying time at 70 °C makes this treatment more efficient while still producing a product with acceptable quality using sensory evaluation.

Hedonic Evaluation

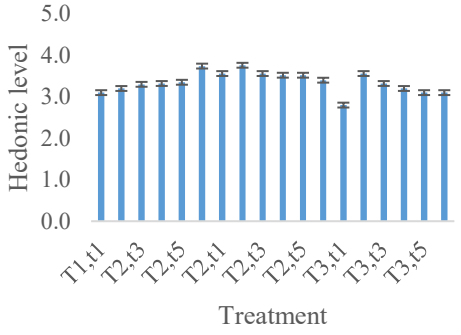
Color

The resulting product retained desirable sensory attributes. Specifically, color evaluation revealed that drying at 70 °C for 4 h produced the most visually appealing tea, characterized by a uniform brown hue that was preferred by the panelists**.** Color evaluation indicated that tea dried at 70 °C for 4 and 6 h exhibited a uniform brown appearance with high panelist acceptance, yielding hedonic scores of 4.92 and 4.85, respectively. There is no significant difference between these two treatments ($p > 0.05$); however, both treatments differed significantly from the other drying conditions (Figure 2a.). However, this treatment was not significantly different from drying at 70 °C for 6 h, suggesting that the shorter 4-hour duration is more efficient while still maintaining acceptable color quality.

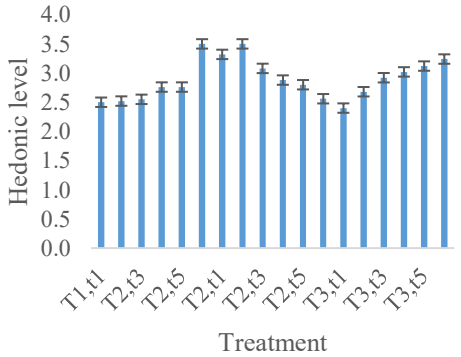
Higher temperatures or longer drying periods tended to excessively darken the tea, whereas shorter drying times resulted in a lighter color that was less attractive. Sensory evaluation was conducted to determine consumer preferences for salak peel tea based on color, aroma, and taste. Color and sensory quality are pivotal attributes in herbal tea development, and extensive research has established that drying parameters significantly influence these characteristics (Mao et al., 2024).



(a)



(b)



(c)

Figure 2. Effect of drying temperature and duration on the hedonic score of salak peel tea taste. Color (a), aroma (b), taste (c).

This study demonstrated that variations in drying temperature significantly altered the color profile of Tencha, with certain settings yielding more desirable visual qualities than others. Similarly, Su et al. (2025) highlighted that drying temperature not only affects the sensory properties of Lichuan black tea, including aroma and taste, but also modulates its bioactive components. These findings revealed that initial drying temperatures played a crucial role in shaping both the biochemical composition and volatile profile of Lishui wild black tea, further underscoring the importance of precise thermal control during processing.

Complementary evidence from herbal tea studies, such as that by Wickramarachchi et al. (2017), confirmed that both temperature and drying duration significantly influenced the nutritional and sensory qualities of Moringa oleifera tea. Collectively, these studies provide strong support for the current investigation, suggesting that optimizing temperature and drying time is essential for improving the visual appeal, nutritional integrity, and consumer acceptance of salak peel tea.

Aroma

Sensory evaluation revealed that drying temperature and duration significantly affected the acceptability of salak peel tea. Samples processed at 60 °C for 12 h had a hedonic score of 3.74, indicating a satisfactory level of panelist approval. A similar result was observed at 70 °C for 4 h, with a score of 3.76, showing that both treatments were well received. Statistical analysis showed no significant difference between the two treatments ($p > 0.05$), suggesting that both drying conditions were similarly well-accepted by the panelists (Figure 2b.). These findings suggest that extended drying at a lower temperature and shorter drying at a slightly higher temperature can produce nearly equivalent outcomes in terms of sensory quality. From a technological perspective, the 70 °C for 4 h treatment may be more efficient, as it maintains high acceptance while considerably reducing processing time and energy requirements.

Recent evidence from studies on herbal and tea products further corroborates the findings of this study. Luo et al. (2025) observed that different drying methods significantly modified the aroma profiles of large-leaf green tea (*Assamica*), and that hot-air drying produced aroma components identified by HS-SPME-GC-MS that were both richer and more preferred in sensory evaluation. Similarly, Bimo et al. (2025) demonstrated that drying at 60 °C for shorter durations enhanced the floral and sweet notes of rose tea while preserving consumer acceptance, even though color intensity decreased under longer drying times or higher temperatures. These parallels suggest that in salak peel tea, as in these teas, moderate temperatures and balanced drying times yield optimal aroma and sensory appeal without compromising visual qualities.

Taste

The taste assessment showed that drying salak peel tea at 60 °C for 12 h resulted in a hedonic score of 3.5, indicating that the product was moderately well accepted in terms of flavor. A similar score of 3.5 was also obtained for the treatment at 70 °C for 4 h, suggesting that the panelists perceived both samples to have comparable palatability. Statistical analysis revealed no significant difference between these two treatments ($p > 0.05$); however, their scores were significantly higher than those of the other drying treatments ($p < 0.05$), demonstrating a superior flavor acceptance (Figure 2c.). This outcome demonstrates that a longer drying process at lower temperatures or a shorter process at higher temperatures can equally preserve desirable taste attributes. Considering processing efficiency, the 70 °C for 4 h condition is advantageous, as it provides the same sensory acceptance level while reducing drying duration.

These results suggest that longer low-temperature drying and shorter moderate-temperature drying are equally effective in preserving desirable taste characteristics of the rice. Comparable outcomes have been reported in studies on other herbal teas. Latarissa et al. (2022) found that drying duration significantly influenced the chemical profile and sensory properties of nutmeg peel tea, where extended drying enhanced certain flavor attributes. Similarly, Bimo et al. (2025)

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demonstrated that drying rose petals at 60 °C for shorter times produced a balanced floral aroma and pleasant taste, whereas higher temperatures reduced flavor acceptability. Bahar et al. (2025) reported that phytochemical composition and subtle differences in taste perception of mixed herbal teas were directly influenced by the drying temperature applied. Together, these findings reinforce the present study, showing that careful adjustment of both temperature and time is essential to optimize the flavor quality of the salak peel tea.

Luo et al. (2025) showed that drying methods significantly altered the aroma and taste profiles of large-leaf green tea, highlighting that controlled hot-air drying better retained pleasant sensory attributes compared to pan-fired techniques. Zhao et al. (2025) further demonstrated that processing methods had a decisive role in determining the flavor of rose herbal tea, where low temperature drying preserved floral and sweet notes more effectively. In addition, Jin et al. (2023) reported that different drying methods strongly influenced the metabolite composition of tea, which was directly associated with differences in taste and overall flavor perception. These findings align with those of the current study, emphasizing that both drying time and temperature must be carefully optimized to achieve a balanced taste and consumer acceptance in salak peel tea.

Proximate Analysis

Proximate composition analysis was performed to assess the nutritional profile of the sample, focusing on moisture, ash, protein, fat, and carbohydrate levels (Table 1). The findings revealed that the sample contained a low level of moisture (3.85 ± 0.026%), which is advantageous for product stability and shelf life, as reduced water content can suppress microbial activity. The ash value was recorded at 5.77 ± 0.039%, indicating the presence of considerable amounts of mineral components. Protein was relatively scarce (0.55 ± 0.001%), indicating that the material is not a major protein contributor. Meanwhile, the fat content reached 3.85 ± 0.001%, which enhanced the flavor and texture.

Table 1. Proximate analysis of salak peel tea

Chemical characteristics	Content (%)
Moisture content	3.85 ± 0.03
Ash content	5.77 ± 0.04
Protein content	0.55 ± 0.00
Fat content	0.06 ± 0.00
Carbohydrate	89.77 ± 0.07

Carbohydrates dominated the composition, accounting for 89.77 ± 0.065%, highlighting the sample as an energy-rich source of carbohydrates. In general, the product is characterized by minimal moisture, appreciable mineral content, and carbohydrates as the predominant constituent. The proximate composition of the dried salak peel tea, characterized by low moisture (3.85 ± 0.026 %) and high carbohydrate content (89.77 ± 0.065 %) with modest ash, protein, and fat, aligns with the nutrient profile typically associated with salak-derived products.

Salacca zalacca fruit is known to have high moisture and carbohydrate content with low protein and fat content, a pattern reflected in its edible fruit composition and potentially in peel-derived products, indicating the carbohydrate-rich nature typical of salak materials (Saleh et al., 2018b). The proximate composition of salak peel tea in this study, which showed a predominance of carbohydrates (89.77%) along with low protein and fat content, aligns with the patterns reported for other herbal teas. For example, a study on *Asystasia gangetica* herbal tea found carbohydrates to be the major nutrient, while protein and fat were detected only at minimal levels (Okolo et al., 2022). Similarly, proximate analysis of fruit tea infusions from Manipur, India, demonstrated that carbohydrates were the principal component, with protein and lipid fractions contributing marginally to the overall composition (Devi et al., 2022). In another study, herbal blends of hibiscus and ginger were evaluated, and although fat levels were relatively low, they played a role in enhancing sensory attributes such as aroma and mouthfeel (Sunday et al., 2024). Moreover, research on composite formulations of *Cymbopogon citratus*, *Lippia multiflora*, and *Ganoderma lucidum* confirmed that low

moisture content is crucial for improving shelf stability and reducing microbial risks in herbal teas (Dzah, 2015). These findings collectively reinforce the observation that plant-based herbal teas are typically rich in carbohydrates, with minor contributions from proteins and fats, and that moisture control is a critical factor for product stability.

Furthermore, community and product development studies highlight the feasibility and market interest in salak peel tea as an antioxidant-rich herbal beverage, reinforcing its potential position in health-oriented markets and local entrepreneurship contexts (Saputri et al., 2024). Finally, the presence of significant phytochemicals such as phenolics and flavonoids in salak peel indicates additional *functional benefits* that complement the proximate nutritive profile, supporting nutritional positioning beyond basic macroanalysis and enhancing its appeal in the functional beverage market (Girsang et al., 2022).

CONCLUSION

These findings indicate that processing temperature and drying duration play a decisive role in shaping the sensory quality of salak peel tea. Treatment at 70 °C for 4 h provided comparable acceptance to 60 °C for 12 h, but with greater efficiency in terms of time and energy use. Nutritional evaluation showed that carbohydrates were the major component, accompanied by low protein and fat contents, while mineral presence was reflected in the ash value. The reduced moisture content further supports the longer shelf stability. When these nutritional and sensory characteristics are viewed alongside existing literature reporting antioxidant and bioactive compounds in salak peel, the product shows potential to be positioned as a functional beverage derived from agricultural byproducts. However, the present study did not directly measure bioactive compounds or antioxidant activity, nor did it involve consumer-based sensory testing, which limits the strength of these functional claims. Therefore, future research should focus on quantifying bioactive components, evaluating antioxidant stability during drying, and conducting broader consumer acceptance studies to more fully support the functional and commercial potential of salak peel tea.

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EFFECT OF BREAD IMPROVER ADDITION ON PREFERENCE, VOLUME EXPANSION, NUTRITIONAL VALUE, AND TOTAL CALORIES OF SWEET BREAD SUBSTITUTED WITH MODIFIED CASSAVA FLOUR (MOCAF)

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ABSTRACT

Modified cassava flour (mocaf) can be used as an alternative to wheat flour to reduce wheat flour consumption. Previous research has shown that substituting wheat flour with mocaf to produce sweet bread with physical and sensory characteristics similar to those of sweet bread made from 100% wheat flour requires a maximum of 20%. Efforts to increase the mocaf substitution to 40% must be accompanied by product modifications, such as the addition of a bread improver, to ensure that the resulting bread remains soft and palatable. This study aimed to determine the effect of adding a bread improver on the preference, volume expansion, nutritional value, and total calories of sweet bread substituted with 40% mocaf. The study was designed using a non-factorial Completely Randomized Design. The amount of bread improver added was 0 g (control), 0.2, 0.4, 0.6, and 0.8 g. The results of the study showed that the addition of bread improvers had a significant effect (p <0.05) (based on the Duncan Multiple Range Test) on total calories, as well as water, ash, protein, and carbohydrate content, and preference for texture, outer color, and inner color of sweet bread (based on the Dunn post hoc test). There was an increase in water, ash, and protein content and a decrease in carbohydrate and total calorie content with increasing amounts of bread improver added to sweet bread, making it a healthier alternative to bread. Meanwhile, the fat content and bread volume expansion were not affected by the amount of bread improver. The panelists liked the color, aroma, texture, and taste of the sweet bread. The addition of a bread improver of 0.8 g / 100 g produced bread that could match the nutritional value and total calories of sweet bread made from 100% wheat flour (without mocaf substitution).

Keywords**:** Bread improver, mocaf, nutritional value, sweet bread, total calories

INTRODUCTION

Rapid population growth has led to an annual increase in wheat flour consumption in China. According to data from the Central Bureau of Statistics, in 2023, Indonesia imported 10,586.6 million tons of wheat seeds (BPS, 2023). Food products made from wheat that are often found in the market include bread, noodles, cakes, and cookies (Zhang, 2020).

The increase in wheat flour consumption in Indonesia is driven by the continued growth of the wheat flour-based product industry, such as bakeries, biscuit factories, and instant noodle manufacturing. This growth indicates that the Indonesian appetite for processed foods made from wheat flour remains high. One effort to reduce the dependence on wheat flour in sweet bread production is to develop substitute ingredients that can reduce imported commodities and utilize local Indonesian commodities, especially in East Kalimantan, namely cassava. Modified cassava flour (MoCAF) is an alternative substitute for wheat flour derived from cassava (Fadilah et al., 2015).

The abundant availability of cassava in East Kalimantan offers an alternative for developing flour substitutes using local commodities. According to data from the East Kalimantan Provincial Food Crops Agriculture Service, cassava production reached 65,167 tons in 2020 (Dinas Pangan,Tanaman Pangan, dan Hortikultara, Kalimantan Timur, 2021). Judging from the

raw materials used to make mocaf, it has good development prospects because of the abundant availability of cassava, and the possibility of a shortage of raw materials can be avoided.

Mocaf flour resulting from yeast fermentation has a water content of 13.14%, ash content of 4.82%, protein content of 1.12%, and fat content of 4.28% (Helilusiatiningsih, 2023), and the carbohydrate content in the form of amylose is quite high, approximately 15-25% (Tako et al., 2014). Research by Yasa et al., (2016) and Nur'utami et al., (2020) found that to produce sweet bread with physical properties (volume expansion) and sensory properties (preference for structure, texture, color, and smell of bread crumbs) that are close to bread without mocaf (100% wheat), the maximum amount of mocaf substituted is only 20%.

This study examined the processing of sweet bread by replacing 40 amount of the wheat flour with mokaf flour. Bread made using mocaf substitution resulted in reduced gluten in the dough, resulting in less carbon dioxide being trapped in the dough during fermentation, resulting in less rise and smaller, denser pores. In this study, bread improver was added with the aim of helping to increase the bread's rising power and the preference for sweet bread, so that it can match or even be better than the quality of sweet bread without mocaf substitution. Bread improvers are additives used to make bread softer and rise better. Bread improvers are made from a mixture of tapioca flour, soy flour, flour treatments (sodium stearoyl lactylate, calcium sulfate), hemicellulose, alpha amylase enzymes, and emulsifiers designed to modify the characteristics of bread dough. The ingredients of this bread improver are thought to affect the nutritional content of sweet bread substituted with mocaf by 40%. Lambert-Meretei et al. (2010) developed a method to evaluate the effect of bread improver dosage and concentration on bread crumb texture. Bread improver (containing lecithin, ascorbic acid, and alpha-amylase) was added to the flour at concentrations of 0.2%, 0.4%, and 0.6%. The hardness, chewiness, stickiness, cohesiveness, and elasticity of the bread were determined using rheological analysis.

MATERIALS AND METHODS

Materials

The basic ingredients used to make sweet bread are high-protein wheat flour (Cakra Kembar brand) and mocaf flour (Ladang Lima brand). Other ingredients included a bread improver (Bakerin Plus brand), which contained a mixture of tapioca flour, soy flour, flour treatment (sodium stearoyl lactylate, calcium sulfate, hemicellulose enzyme, and alpha amylase enzyme), instant yeast (Fermipan brand; the yeast to be used was ensured to be active by mixing 3 g of yeast and 30 g of sugar into 20 g of water, then letting it stand for 10 min until it foams or bubbles; if foam forms, it means the yeast is active), granulated sugar (Gulaku brand), butter (Hollman brand), full cream milk powder (Dancow brand), chicken eggs, and salt. The chemicals used for proximate analysis were sulfuric acid, 10% sodium hydroxide, 0.02 N hydrochloric acid, catalyst (containing sodium sulfate, copper sulfate, and selenium), petroleum benzine, saturated boric acid, methylene red, and methylene blue. All chemicals were obtained from Merck (Darmstadt, Germany).

Experimental Design

This study used a non-factorial completely randomized design consisting of five levels of bread improver amount treatment, each carried out with three replications. The bread improver amount treatments consisted of: control (0.0 g), 0.2, 0.4, 0.6, and 0.8 g. was made from wheat flour and mocaf flour at a ratio of 60:40. The total raw material of the flour mixture per treatment was 100 g. For comparison, sweet bread (PM) was prepared using 100% wheat flour without the addition of mocaf and without the addition of bread improver.

Research Parameters

The parameters tested in this study were preferences (hedonic test) for the color, aroma, taste, and texture of the bread, the bread volume expansion test, the proximate test (nutritional value), and the total calorie content of the bread. The hedonic test for the color, aroma, texture, and taste of sweet bread substituted with mocaf used 25 untrained panelists, following the method suggested by

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Setyaningsih et al. (2010). Bread volume expansion testing was performed according to the method suggested by Wardani et al. (2012), with some modifications. Proximate tests covering water, ash, fat, protein, and carbohydrate content were performed according to the method described by AOAC (2019), and total calorie testing using the Atwater factor was performed according to Almatsier (2002).

Data Analysis

Sensory hedonic test data were analyzed using the non-parametric Friedman test. If the test results showed a significant difference between the treatments, a Dunn test was performed. Data from the bread volume expansion test, proximate test, and total calories were analyzed using a one-way analysis of variance (one-way ANOVA) test, with a further test using Duncan's Multiple Range Test (DMRT). If the data distribution shows that it is not normal or/and not homogeneous, the test was switched to using the Kruskal-Wallis test, which was followed by a Tukey difference test if there was a significant difference between treatments.

Processing of Sweet Mocaf Bread

Sweet Mocaf Bread (SMB) processing for each treatment uses raw materials of high protein wheat flour (60 g), mocaf flour (40 g), granulated sugar (30 g), full cream milk powder (12 g), instant yeast (3 g), 1 g salt, bread improver (BI) (the amount of BI according to the treatment is 0.0, 0.2, 0.4, 0.6, and 0.8 g). All dry ingredients were placed in a dry container and stirred with a spatula until evenly mixed. Next, a mixture of 30 g of chicken egg yolk and white was added and stirred evenly using a mixer, starting with low to high speed until a semi-smooth texture was obtained. Then, 15 g of butter was added, and stirring was continued using a mixer at high speed. During the stirring process, sufficient water is required to form a dough until it becomes smooth. Next, the first fermentation process was carried out for 60 min, after which the dough was deflated, and continued by weighing the dough was weighed as much as 50 g each. The dough was shaped into balls and placed on a baking sheet lined with the parchment paper. The dough was then proofed for 30 min. The dough was baked in an electric oven at 170 °C for 25 min or until cooked through. The comparison bread (PM) in this study was an original sweet bread made with 100 g of wheat flour, without the addition of mocaf and BI, while the other ingredients were the same as in the SMB.

RESULTS AND DISCUSSION

Sensory Hedonic Characteristics (Preference) on Sweet Mocaf Bread

The recapitulation of the data from the results of the preference test for color, aroma, texture, and taste of sweet bread substituted with mocaf is presented in Table 1.

Table 1. Effect of the amount of bread improver (BI) on the sensory hedonic characteristics of sweet mocaf bread

BI (g)	Color		Aroma	Texture	Taste
	Outer of bread	Inside of bread			
0.0	3^{a}	4^{a}	4	4^{a}	4
0.2	4^{ab}	4^{ab}	4	4^{b}	4
0.4	4^{a}	4^{ab}	4	4^{bc}	4
0.6	4^{a}	4^{ab}	4	4^{ac}	4
0.8	4^{b}	4^{ab}	4	4^{a}	4
PM	4^{b}	4^{b}	4	4^{b}	4

Note: Data (median) were obtained from 75 responses. Data were analysed by Friedman test. Data within the same column followed by different letter showed a significant difference (Dunn's test, $p<0.05$). PM: comparison of sweet bread made from 100% wheat flour, without mocaf substitution, and without the addition of BI (bread improver). Preference score: 1=dislike very much, 2=dislike, 3=like somewhat, 4=like, 5=like very much

The addition of BI did not significantly affect the panelists' preference for the aroma of the control, SMB, and PM samples. All treatments were preferred by the panelists (score: 4). The formation of bread aroma is more influenced by the basic ingredients used, such as yeast, which produces enzymes that ferment/convert compounds that produce alcohol and carbon dioxide, resulting in the distinctive aroma of bread will be formed due to the formation of acids, aldehydes, and esters. Although mocaf flour has a distinctive aroma, this aroma is masked by the use of other ingredients such as milk, butter, and eggs, which have stronger aromas. This is what makes the bread smell distinctly different.

The panelists preferred (score 4) the texture of the sweet bread in all SMB and PM treatments. With the addition of 0.6 g BI, the texture began to become slightly sandy, and with the addition of 0.8 g BI, the texture was very brittle and sandy/crumbly. This extremely brittle and sandy/crumbly texture indicates that the composition exceeds the recommended usage limit. This is in accordance with Kusnedi (2021), who reported that using the recommended composition, BI could work optimally without disrupting the function of other ingredients. However, if the amount is less than the recommended composition, there is a high possibility that the bread will be dense, and if it exceeds the upper limit of use, the gluten chain will be damaged, and the bread dough structure will become brittle.

There was no significant difference in taste preferences among all treatments, and the panelists preferred the taste of SMB (score 4). Based on research by Kartikasari et al. (2019), the use of BI does not interfere with the taste of the resulting bread; in fact, the addition of BI helps improve and balance the taste of sweet bread. The combination of enzymes (alpha amylase, hemicellulose, and protease) in the BI composition breaks down starch into simple sugars, which are a food source for yeast fermentation, producing gas, which also increases the sweetness of bread (Kusnedi, 2021). In addition, BI can help control the acidity level in bread by balancing the fermentation and baking processes, which reduces the overly strong sour taste that can appear in bread fermented for too long.

Volume Expansion of Bread

The data from the volume expansion test of sweet bread substituted with mocaf (SMB)and the comparison bread (PM) are presented in Table 2.

Table 2. Effect of bread improver on volume expansion of sweet bread substituted with mocaf

Bread improver (g)	Volume expansion of SMB (%)
0.0	37.10±0.26[a]
0.2	39.09±1.47[a]
0.4	40.59±1.72[ab]
0.6	42.96±1.38[ab]
0.8	44.19±1.83[ab]
PM	47.54±1.63[b]

Notes: Data (mean ± SD) obtained from three replicates. Data were analyse by Kruskal-Wallis test. Data within the column followed by different letter showed significant differences (Tukey test, $p<0.05$). PM: Sweet bread made from 100% wheat flour, without mocaf substitution and without the addition of bread improver (BI).

The addition of BI significantly affected the expansion power of the resulting SMB. The increase in the amount of BI showed an increase in the volume of SMB, which ranged from (37.10% to 44.19%. PM bread had the highest volume expansion, namely 47.54%. The increase in the amount of BI made the dough more elastic to trap CO_2 gas produced during fermentation by yeast, allowing the dough to expand and form pores. (Rosalina et al., 2018). Penelitian terdahulu yang dilakukan oleh Previous research conducted by Nur'utami et al. (2020) howed that the higher the percentage of mocaf flour added, the lower the rise power of sweet bread. This is because mocaf does not contain gluten protein, which can form dough elasticity, increase the CO_2 capture capacity of yeast fermentation, and form the dough structure (Putri, 2023). Therefore, by adding BI, which contains a combination of alpha amylase and hemicellulose enzymes, it functions to cut the starch in the bread dough into simple sugars, which are a food source for

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yeast for fermentation, producing gas, which increases the expansion of the bread volume.

Nutritional Value And Total Calories

The proximate testing results of sweet bread substituted with mocaf, including water, ash, fat, protein, carbohydrate, and total calorie content, are presented in Table 3.

The addition of different amounts of BI significantly affected the SMB moisture content. The moisture content of the sweet bread ranged from 15.40 to 23.00%. These test results still meet the bread quality standards according to SNI 01-3840-1995, which is a maximum of 40%. Making bread using mocaf flour requires the addition of ingredients that can help bind the starch granules to maintain the attractive force between the starch granules and the gas in the dough. (Koswara, 2009). Bread improvers (BI) bind starch granules in dough, ensuring proper gas retention during fermentation. Furthermore, BI contains the enzyme amylase, which breaks down starch into smaller molecules, helping the yeast absorb water and increase gas production in the dough (Chen et al., 2021), and emulsifiers that can strengthen gluten and increase the ability of amylose to retain moisture, thus allowing bread dough to retain more water and produce bread that rises and has a good texture.

Table 3. Effect of the amount of bread improver (BI) on the nutritional value and total calories of sweet bread substituted with mocaf (SMB)

Bread improver (g)	Water content (%)	Ash content (%)	Protein content (%)	Fat content (%)	Carbohydrat content (%)	Calories (kkal/100 g)
P1 (0,0)	15.41±0.18^{a}	1.56±0.04^{a}	9.07±0.06^{a}	11.19±0.20	62.76±0.21^{f}	388.11±1.25^{e}
P2 (0,2)	16.26±0.11^{b}	1.85±0.01^{b}	9.22±0.04^{b}	11.26±0.12	61.41±0.14^{e}	383.87±0.89^{d}
P3 (0,4)	17.44±0.14^{c}	2.35±0.05^{c}	9.53±0.08^{c}	11.38±0.19	59.29±0.18^{d}	377.79±1.29^{c}
P4 (0,6)	18.48±0.14^{d}	2.94±0.04^{d}	9.75±0.10^{d}	11.39±0.20	57.42±0.24^{c}	371.28±1.38^{b}
P5 (0,8)	20.37±0.07^{e}	3.34±0.03^{e}	10.02±0.04^{e}	11.58±0.20	54.69±0.11^{b}	363.09±1.35^{a}
PM	23.00±0.50^{f}	1.48±0.19^{a}	13.52±0.13^{f}	11.80±0.30	50.43±0.94^{a}	361.10±1.53^{a}

Notes: Data (mean ± SD) were obtained from three replicates. Data were analysed by ANOVA. Data within the same column followed by different letter indicate a significant difference (DMRT, $p<0.05$). PM: sweet bread made from 100% wheat flour without mocaf substitution and without the addition of bread improver.

The ash content of the SMB increased with the amount of BI used. Bread improvers also contain minerals such as magnesium, calcium, and potassium. These minerals come from the raw materials in the BI content, such as soybean flour, tapioca, and calcium sulfate. This can enhance the interaction between minerals in the bread dough, resulting in complex compounds that contribute to the increase in the ash content of sweet bread substituted with mocaf.

The protein content of SMB increased with an increase in BI. The protein content of bread is influenced by the type of flour used and the added protein sources, such as milk, eggs, and other additives (Nuraisyah et al., 2018). Mocaf flour has a much lower protein content than high-protein wheat flour, which has approximately 12-14% protein. (Abidin et al., 2013). Therefore, substituting 40% wheat flour with mocaf flour reduced the overall protein content of the sweet bread. In sweet bread preparation, mocaf substitute flour enhances gluten network formation, thereby retaining moisture and improving the dough structure. This improved dough structure allows the proteins from the ingredients to mix evenly and bind tightly within the dough (Kartikasari et al., 2019). Bread improvers contain protein derived from soybean flour, malt, and tapioca flour, although the amounts are small. Therefore, using more BI can increase the protein content of sweet bread substituted for mocaf by 40%.

The fat content of SMB ranged from 11.19% to 11.58%. The addition of BI did not significantly affect SMB fat content. This is because the added BI was in small amounts and contained only a small amount of fat derived from soy flour and tapioca flour. Meanwhile, the largest amount of fat in SMB comes from the ingredients used in making

sweet bread, such as eggs, milk, butter, and flour. Therefore, overall, BI did not affect the fat content of SMB. The use of ingredients such as eggs, butter, and milk is important for producing sweet bread that tastes delicious with a soft and fragrant texture (Male et al., 2017). The fat content of the sweet bread was higher than the maximum sweet bread quality standard according to SNI 01-3840-1955, which is 3. The fat content of SMB was also not significantly different from that of PM bread.

The carbohydrate content of SMB ranged from 54.69 to 62.76%. Other nutritional components can also affect the carbohydrate content calculated using the by-difference method (Riskiani et al., 2014). Differences in the carbohydrate content of ingredients, such as flour, also affect the carbohydrate content of sweet bread (Hastian and Basir, 2022). The carbohydrate content of SMB decreased with increasing amounts of added BI and was higher than that of PM bread. Mocaf contains approximately 87.3% carbohydrate, whereas wheat flour contains approximately 60-68% carbohydrate (Dwipayanti et al., 2020).

The total calories of SMB ranged from 363.09 to 388.11 kcal, which was higher than the total calories of PM bread (361.10 kcal). The total calorie content of sweet bread is influenced by nutritional components such as fat, protein, and carbohydrates. The energy obtained from food products is usually expressed in calorie units. The total calories were determined using an empirical calculation method by converting nutritional components, such as fat, protein, and carbohydrates, into kilocalorie units by multiplying these components by a conversion factor. The conversion factors for fat, protein, and carbohydrates are 9, 4, and 4 kcal, respectively (Almatsier, 2002). The higher the amount of BI used in making sweet bread as a mocaf substitute, the lower the total calories of the sweet bread produced. This is because BI increases the functional properties of flour, thereby reducing the need for high-calorie ingredients, such as fat and additional sugar, in the process of making sweet bread.

CONCLUSION

The addition of bread improver had a significant effect ($p<0.05$) on the preference for texture, outer color, inner color, volume expansion, water content, ash content, protein content, carbohydrate content, and total calories of sweet bread with mocaf. There was an increase in water, ash, and protein content and a decrease in carbohydrate and total calorie content with increasing amounts of bread improver added to sweet bread, making it a healthier alternative to bread. Meanwhile, the fat content and bread volume expansion were not affected by the amount of bread improver. From a sensory perspective, sweet bread was preferred for outer color, inner color, aroma, texture, and taste, equaling the preference for sweet bread without mocaf substitution and without the addition of bread improver. The addition of a bread improver at 0.8 g / 100 g produced bread that matched the nutritional value and total calories of sweet bread made from 100% wheat flour (without mocaf substitution).

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OPTIMIZATION OF DRYING AND BREWING CONDITIONS OF CASCARA FOR KOMBUCHA PRODUCTION BASED ON SENSORY CHARACTERISTICS

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ABSTRACT

Indonesia is among the largest global coffee producers, with an output of 786.19 thousand tons in 2021, largely contributed by smallholder coffee plantations. Coffee fruit is composed of approximately 40% beans and 45% cascara, the latter of which is often discarded and underutilized despite its high polyphenol content. Improper disposal of cascara may cause environmental problems because of its acidity and antinutritional compounds. Cascara represents a promising raw material for functional beverages, such as kombucha, to increase its antioxidant potential. This study focused on developing cascara kombucha from coffee harvested in Magelang, Central Java, a region with distinct geographical characteristics that influence coffee quality. The cascara was dried at different temperatures (35, 40, and 45 °C for 24 h), brewed for 5 and 10 min, and subsequently fermented with a 10% (w/v) kombucha SCOBY for 8 d before pasteurization and storage. Sensory evaluation was performed by trained panelists using Quantitative Descriptive Analysis, followed by a hedonic acceptance test with 26 untrained panelists. The results demonstrated that drying at 45 °C combined with 5 min brewing yielded the highest hedonic score (4.88), while drying at 35 °C with 5 min brewing provided a comparable result (4.73) without significant difference. A longer brewing time (10 min) increased the turbidity. Optimization using DX13 analysis indicated that drying at 35 °C for 24 h and brewing for 5 min achieved the highest desirability for more effective production. These findings suggest that cascara kombucha has potential as a novel functional beverage, although further optimization is required to maximize its antioxidant and phenolic retention.

Keywords: brewing, cascara, drying, kombucha, optimization

INTRODUCTION

Coffee production in Indonesia has shown an increasing trend annually. In 2021, production reached 786.19 thousand tons, representing a 3.12% increase compared to the previous year, with the majority originating from smallholder plantations that account for 99.32% of the total coffee cultivation area in the country (Badan Pusat Statistik, 2022). Domestic coffee consumption has also experienced positive growth, with the highest annual growth rate of 1.7% during 2017–2021, surpassing the global coffee consumption growth rate of only 1.0% (International Coffee Organization, 2021). This condition reflects the growing interest of Indonesian society in coffee consumption, in line with the increase in its production.

Coffee fruit consists of approximately 45% peel (cascara), 10% mucilage, 5% parchment skin, and 40% coffee beans (Nurhayati et al., 2020). Despite being the largest component, the peel or cascara is often discarded during commercial coffee processing, as only the beans are used. Cascara has mainly been used as a compost, bioethanol, and animal feed. Nevertheless, this waste can cause considerable environmental problems. Compounds such as caffeine, polyphenols, and tannins in cascara contribute to environmental pollution in coffee-producing countries (Anal, 2017). The acidic nature of coffee peels and mucilage may also cause equipment corrosion and complicate disposal processes. If released into

aquatic environments, waste can lower water pH and endanger fish and other aquatic organisms (Riandani et al., 2022). Furthermore, the utilization of cascara as animal feed is limited because of the presence of caffeine and tannins, which act as antinutritional factors (Esquivel and Jimenez, 2012).

Although cascara has several negative effects, it contains high levels of polyphenols that are beneficial as antioxidant compounds for humans. Antioxidants play a role in scavenging free radicals that accumulate in the body, thereby helping to prevent degenerative diseases, such as cancer and diabetes (Laila et al., 2024). Recently, the use of cascara as a functional beverage ingredient has gained increasing attention, for instance, in the form of cascara tea prepared similarly to conventional tea. This beverage has gained popularity in several coffee-producing countries. Cascara infusion produces a reddish-brown color similar to that of tea (Judkis, 2017), with a fruity aroma described as a combination of blackcurrant and watermelon (Pabari, 2014). Other studies have reported cascara aromas resembling rose, cherry, mango, and tobacco (Muzaifa et al., 2019).

The antioxidant capacity of cascara is influenced by its phenolic content, including protocatechuic acid, chlorogenic acid (Heeger et al., 2017), and hydroxycinnamic acids (Cubero-Castillo et al., 2017). To date, cascara has been processed using brewing methods. One alternative approach is to ferment cascara into kombucha tea. Kombucha is a fermented beverage prepared from brewed leaves or fruits with added sugar, followed by fermentation with a starter culture known as the Symbiotic Culture of Bacteria and Yeasts (SCOBY) (Nurhayati et al., 2020).

The fermentation process, which typically lasts 1–2 weeks, can influence the sugar content, alcohol content, pH, and antioxidant capacity. During fermentation, *Saccharomyces cerevisiae* breaks down glucose into ethanol, whereas *Acetobacter xylinum* oxidizes ethanol into acetic acid. Microbial enzymatic activity during fermentation may also enhance the levels of free polyphenols, as enzymes release bound polyphenols, making them more detectable (Zubaidah et al., 2012). However, only a small fraction (5–10%) of dietary polyphenols is absorbed by the human body. Unabsorbed polyphenols reach the large intestine, where they interact with the gut microbiota. Rodríguez-Daza et al. (2021) highlighted the dual action of polyphenols, which can stimulate probiotic bacterial growth while suppressing pathogenic bacterial populations.

The physical and chemical characteristics of coffee fruits vary across regions owing to geographical differences. According to Hu et al. (2024), higher elevations tend to produce coffee with chemical compositions that are associated with more complex flavors. Magelang Regency is characterized by a basin landscape surrounded by five mountains, including Mount Sumbing. Coffee in this region is cultivated at altitudes ranging from 900 to 2,000 m above sea level (Direktorat Jenderal Kekayaan Intelektual, 2023). Since 2016, the Ministry of Agriculture has designated Magelang as a national priority area for coffee plantation development (Kementerian Hukum dan Hak Asasi Manusia Republik Indonesia, 2022). With these distinctive geographical conditions, coffee from Magelang is assumed to have unique flavor profiles and physicochemical properties.

In kombucha preparation, cascara must be dried to facilitate the brewing process. Proper control of temperature and time during brewing is essential to ensure the safety of the product. However, heat application during drying and brewing may affect polyphenol content, as these compounds possess chemical structures that are highly susceptible to thermal degradation, which can reduce antioxidant activity (Kurniati et al., 2019; Dewi et al., 2022). Furthermore, the drying temperature significantly influences the aroma and color quality of cascara tea infusions (Tampubolon et al., 2024), which subsequently undergo fermentation.

To date, studies specifically investigating the effects of drying temperature and pasteurization on the sensory characteristics of cascara kombucha, particularly from Magelang coffee, are limited. The present study builds upon a previous study that compared Arabica coffee cherries with and without sorting. The

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findings indicated that the sensory attributes of the two products did not differ significantly from those of commercial kombucha. However, heating and drying processes have been shown to reduce the antioxidant and phenolic contents of kombucha (Gunawan et al., 2025). Therefore, determining the appropriate drying and pasteurization temperatures is necessary to obtain cascara kombucha with optimal antioxidant activity.

MATERIALS AND METHODE

Material

The materials used in this study included cascara, SCOBY (Symbiotic Culture of Bacteria and Yeast), and granulated sugar. Cascara was obtained from coffee harvested by farmers in the Windusari District, Magelang Regency. The cascara used consisted of sorted peels from ripe coffee cherries, whereas cascara from unripe green cherries was discarded. Prior to use, the cascara was thoroughly washed, packed in ziplock plastic bags, stored in a freezer, and subsequently dried using a dehydrator at the designated treatment temperature. SCOBY was purchased online from a local kombucha culture supplier and used as a fermentation starter. Granulated sugar was sourced from a local market in Magelang and served as the sucrose source during the kombucha fermentation process.

Experiment Design and Analysis

This study employed a 3×2 factorial experimental design with two treatment factors: cascara drying temperature (35, 40, and 45 °C) and brewing time (5 and 10 min), resulting in six treatment combinations (35-5, 35-10, 40-5, 40-10, 45-5, and 45-10). Each treatment combination was replicated twice, yielding 12 samples. The observed variables included pH, total soluble solids (°Brix), and sensory characteristics assessed through Quantitative Descriptive Analysis (QDA) and an overall hedonic test. This design was used to evaluate the effects of drying temperature and brewing time on the physicochemical and sensory qualities of cascara kombucha. Data optimization analysis was performed using Design Expert 13 software with the Response Surface Method, while sensory test data were analyzed using ANOVA followed by Duncan's and Dunnett's post-hoc tests at a significance level of 0.05.

Research Prosedure

Postharvest Handling of Coffee Cherries

Coffee cherries were first sorted based on quality and ripeness, with unripe green cherries being discarded. Cascara from ripe cherries was then washed and stored in ziplock plastic bags in a freezer to prevent the loss and evaporation of the bioactive compounds. Subsequently, the cascara was dried using a dehydrator at 35, 40, and 45 °C for 24 h. The dried cascara was then repackaged in zip-lock plastic bags with silica gel and stored at room temperature.

Preparation of Cascara Kombucha

Cascara tea was prepared by mixing 1% (w/v) dried cascara and 10% (w/v) sucrose in 3 liters of water, following the method described by Nurhayati et al. (2020). The solution was boiled and maintained for 5 and 10 min according to the treatment variation. The cascara infusion was subsequently transferred into glass jars and allowed to cool overnight at room temperature. Thereafter, the infusion was inoculated with 10% (w/v) kombucha SCOBY and fermented for 9 days, with pH measurements taken every 3 days. The fermented cascara solution was then separated from the culture, pasteurized at 72 °C for 15 s, and bottled in tightly sealed 250 mL plastic containers for further analysis.

Analytical Procedures

Physicochemical Analysis

pH testing was conducted to determine the acidity level of kombucha throughout fermentation and in the final product, as pH is a key indicator of the safety and stability of fermented beverages. Measurements were performed using a calibrated pH meter with pH 7 and pH 4 buffer solutions, following Puspaningrum et al. (2022). Total soluble solids were measured to determine the concentrations of dissolved compounds, such as sugars, organic acids, and bioactive fermentation products. The analysis was carried out using a manual refractometer, expressed in °Brix, by placing a drop of sample on the prism and reading the value from the optical scale.

Sensory Evaluation

Sensory evaluation of cascara kombucha was conducted using the QDA method, with parameters including aroma, texture, color, and taste, to assess descriptive sensory characteristics. The evaluation involved six trained panelists and 26 untrained panelists recruited from students of the Department of Food Technology. Additionally, a hedonic test was conducted to assess consumer acceptance of the product, involving the same 26 untrained panelists. A seven-point overall hedonic scale was employed, ranging from "Dislike Extremely" to "Like Extremely." The panelists completed the evaluation forms based on their individual responses. Water was provided between the samples to neutralize taste perception.

RESULTS AND DISCUSSION

Physicochemical Analysis

The results of the total soluble solids (°Brix) test under the different drying and brewing treatments are presented in Figure 1.

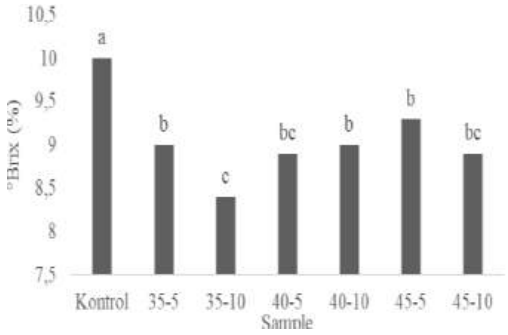

Figure 1. Effect of drying and brewing on total soluble solids (°Brix) of cascara kombucha. Bars followed by the same letters indicate no significant difference (DMRT, α = 5%). Cascara drying temperature was 35, 40 and 45 ºC, while the brewing time were 5 and 10 min.

The control sample recorded a total soluble solids value of 10.0%, whereas cascara kombucha after fermentation showed reduced values ranging from 8.4% to 9.3%. This reduction indicates that fermentation by the microbial consortium of kombucha (SCOBY) utilized simple sugars as the main substrate, thereby decreasing the total soluble sugar concentration in the medium. These findings are consistent with Shafira et al. (2022), who reported that cascara kombucha fermentation consistently decreases °Brix with increasing fermentation time.

Analysis of variance (ANOVA), followed by Duncan's test (α = 0.05), revealed three homogenous groups of °Brix values. The 35–10 treatment (8.4%) formed a distinct group with the lowest value, which was significantly different from that of the control (10.0%). Treatments 40–5 (8.9%) and 45–10 (8.9%) overlapped with the intermediate group (9.0–9.3%), showing no significant difference from treatments 35–5 (9.0%), 40–10 (9.0%), and 45–5 (9.3%). The control (10.0%) was classified into a separate subset with the highest value. Accordingly, only the 45–5 treatment (9.3%) approached the control, although it remained significantly different from it.

The lowest °Brix value (8.4%) in the 35–10 treatment can be attributed to prolonged brewing at a lower drying temperature, which likely extracted more sugars and bioactive compounds, enriching the initial substrate and enabling more intensive fermentation. Conversely, the 45–5 treatment, with a higher drying temperature and shorter brewing time, yielded a higher post-fermentation °Brix value (9.3%) because fewer soluble sugars were initially extracted, thereby limiting the microbial sugar consumption. Higher drying temperatures (40 °C and 45 °C) consistently produced higher °Brix values (8.9–9.3%) than the 35–10 treatment, suggesting that the availability of soluble sugars in the substrate was limited from the outset. This may be due to the degradation of simple sugars during the high-temperature drying process. Another plausible explanation is that high-temperature drying reduced the cascara moisture content, causing sugars initially bound to water to revert to the solid phase. These sugars require a longer time to dissolve during brewing, thereby restricting fermentation intensity and preventing drastic °Brix reductions compared to low-temperature drying.

These findings are consistent with those of Martínez-Soto et al. (2023), who reported that postharvest treatments, such as size reduction, affect the content of simple sugars and bioactive compounds in cascara. Villarreal-Soto et al. (2018) highlighted that

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the availability of initial substrates is a key determinant of kombucha fermentation intensity. Furthermore, Tampubolon et al. (2024) observed that higher drying temperatures significantly ($p < 0.01$) reduced moisture and polyphenol content, supporting the hypothesis that thermal drying may reduce the amount of extractable solutes in the initial stage.

The results of the pH measurements during fermentation under different drying and brewing treatments are presented in Figure 2.

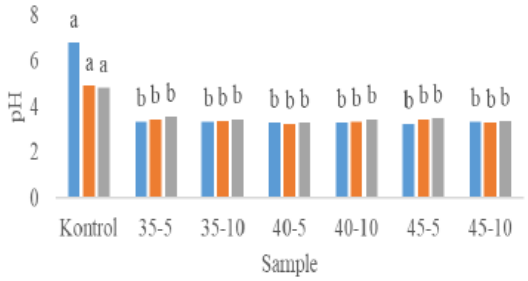

Figure 2. Effect of drying and brewing on the pH of cascara kombucha. Bars followed by the same letters indicate no significant difference (Dunnett's test, α = 5%). Blue, orange, and grey bars represented the 3rd, 6th, and 8th d of brewing. Cascara drying temperature was 35, 40 and 45 °C, while the brewing time were 5 and 10 min

Dunnett's test confirmed that drying, brewing, and fermentation treatments significantly altered the pH compared to the control (untreated). The pH of the control sample decreased only to 4.8 by day 8, whereas the pH of the fermented treatments rapidly decreased to a range of 3.3–3.6 by day 3. This significant reduction highlights that active microbial fermentation in kombucha was the primary driver of organic acid production (mainly acetic, gluconic, and lactic acids), a process that was absent in the control (Jayabalan et al., 2014; Villarreal-Soto et al., 2018).

These results reinforce the role of cascara fermentation in lowering pH to levels that ensure microbiological stability (<4.0), making the product safer for storage than the control (Marsh et al., 2014). The sharp decrease in pH in fermented cascara kombucha was primarily caused by the production of organic acids, particularly acetic and gluconic acids, by the symbiotic microbial community of acetic acid bacteria and yeasts (Villarreal-Soto et al., 2018). The availability of simple sugars extracted from cascara through drying and brewing supports this fermentation activity. Consequently, all fermentation treatments exhibited consistently low final pH values (3.3–3.6), indicating optimal fermentation. These results are consistent with those of Jayabalan et al. (2014), who reported that kombucha fermentation decreases the pH to 2.5–3.5 within 7–10 days owing to organic acid accumulation.

Although different drying temperatures and brewing times produced variations in the final °Brix values (as previously discussed), the pH values were relatively uniform across the treatments. This suggests that despite differences in initial sugar availability, kombucha microbes consistently reduced pH to similar levels, likely due to the buffering effect of the fermentation system and uniform activity of acetic acid bacteria under the given substrate conditions.

Sensory Evaluation

The results of the sensory analysis are presented in Figure 3.

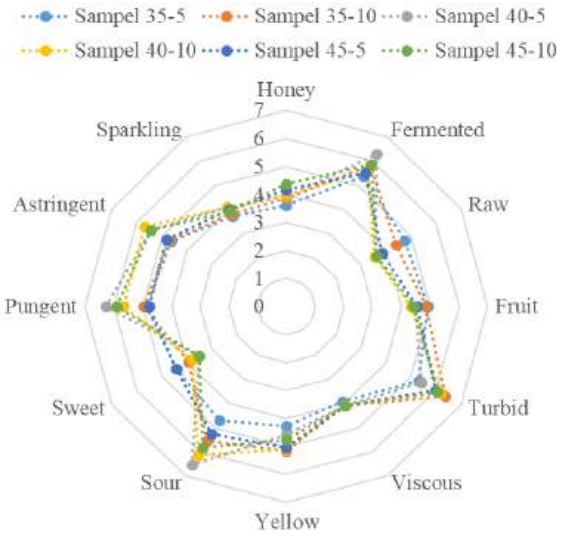

Figure 3. Spider web diagram showing the effects of drying and brewing on the sensory quality of cascara kombucha. Cascara drying temperature was 35, 40 and 45 °C, while the brewing time were 5 and 10 min.

Quantitative Descriptive Analysis (QDA) is one of the most widely applied sensory descriptive methods for evaluating the sensory profiles of food products. Trained panelists assess the intensity of predefined sensory attributes, such as aroma, taste, aftertaste, texture, and visual characteristics. Evaluations are performed using numerical intensity scales (e.g., 0–10), allowing for the quantitative measurement of complex sensory perceptions (Stone et al., 2020). This method is regarded as reliable and reproducible because it relies on trained panelists who undergo calibration, thereby minimizing individual biases. QDA has been extensively applied in studies of fermented products, including tea, coffee, and kombucha, to assess the influence of processing on consumer perceptions (Lawless & Heymann, 2010).

QDA results are commonly presented in spider web (radar) diagrams, which depict the intensity of each attribute on a circular coordinate plane. Each axis represents a sensory attribute, and intensity values are plotted by distance from the center, facilitating intuitive visualization of differences among treatments.

Analysis of the spider web diagram using ANOVA followed by Duncan's test revealed that the "Honey" aroma was most prominent in the 45–10 treatment, significantly different from the 35–5 treatment, which, together with 35–10, showed stronger "Raw" aroma. Extended drying and brewing periods tended to increase the intensity of the honey-like aroma while diminishing the raw aroma attributes in cascara kombucha. Samples brewed for 10 min exhibited distinct sensory traits, namely turbidity (opaque texture), enhanced yellow color intensity, and increased astringency. Prolonged brewing has been reported to increase turbidity in tea-based beverages (Wang et al., 2021) and extract polyphenolic compounds that contribute to a pronounced aroma and taste (Su et al., 2025; Guo et al., 2021).

Interestingly, the 40–5 treatment produced the most intense and sharp flavor profile, characterized by a strong fermentative aroma, acidity, and pungency. Samples brewed for 10 min also exhibited high values for these attributes, although the 40–5 treatment remained superior. Conversely, sweetness was more pronounced in the samples brewed for 5 min, except for 40–5. These results were not fully consistent with the °Brix analysis shown in Figure 1. Although the 35–10 treatment had the lowest total soluble solids (suggesting the highest fermentation activity), its sensory fermentation attributes were less intense than those of 40–5. However, the °Brix values of these two treatments after fermentation were not significantly different from each other. Similarly, the lowest sweetness intensity was expected in 35–10, given its lowest °Brix, yet QDA indicated that 40–5 had the lowest perceived sweetness. Based on sensory analysis, the 40–5 treatment exhibited the most intensive fermentation activity. At 40 °C drying and 5 min brewing, it is plausible that soluble solids other than sugars were extracted more effectively, resulting in a higher °Brix than 35–10.

Optimization analysis using Design Expert 13 (DX13) was performed considering temperature and time are within the range (possibility of recreating the production using the tools stated in this study), the highest overall hedonic scores resulting in a cascara kombucha product liked by the consumers, minimum turbidity to produce clarity of the product, and low pH as indicators of food safety (to make cascara kombucha a low-risk product, as pathogenic and spoilage microbes will have difficulty surviving in an acidic pH). The hedonic test results showed that the most preferred sample was produced by drying at 45 °C and brewing for 5 min, although its score was not significantly different from most treatments, except for 40–5, which had the lowest acceptance. The minimum turbidity was recorded for the 35–5 treatment, whereas all treatments yielded comparably low pH values.

Response Surface Methodology (RSM) was employed as a statistical and mathematical approach to model and analyze the effects of multiple independent factors on one or more response variables. The primary aim was to identify the optimal factor combinations that yield the most desirable responses (Montgomery, 2017). The experimental design was set using the custom design option, followed by an analysis to

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determine the solution with the highest desirability value. DX13 suggested drying at 35 °C with 5 min boiling as the optimal condition, predicting an overall hedonic score of 4.4, minimum turbidity of 5.240, and a safe pH of 3.442, with a desirability index of 0.553. However, these predicted values require further validation in future studies.

CONCLUSION

This study confirmed that the drying and brewing methods of cascara significantly influenced the physicochemical and sensory characteristics of the resulting kombucha. Sensory analysis revealed distinct differences in profiles across treatments, and statistical tests confirmed that not all treatment conditions yielded results comparable to those of the control. Furthermore, optimization using Response Surface Methodology (RSM) recommended a drying temperature of 35 °C combined with a brewing time of 5 min as the optimal condition, providing a balance between sensory quality, safety, and consumer preference. These findings highlight the importance of selecting appropriate processing conditions to produce high-quality cascara kombucha with potential for industrial-scale production.

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DEVELOPMENT AND NUTRITIONAL EVALUATION OF CRACKERS FORTIFIED WITH YELLOWSTRIPE SCAD (*Selaroides leptolepis*) WHOLE POWDER AS A FUNCTIONAL FOOD FOR STUNTING PREVENTION

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ABSTRACT

Yellowstripe Scad (*Selaroides leptolepis*) has potential as a fortificant to enhance the nutritional quality of crackers. The incorporation of yellowstripe scad whole fish powder not only improves the nutrient content but also reduces processing waste. Fish-based crackers can serve as dietary food products, nutrient-dense snack alternatives, and supplementary foods to support stunting prevention. This study aimed to evaluate the nutritional composition (moisture, lipid, dietary fiber, protein, and ash content) and physical quality (texture) of fish crackers. The experiment was designed using a Completely Randomized Design with a single factor, namely, the addition of yellowstripe scad whole powder (SFF) at six levels: 0, 3, 6, 9, 12, and 15%. The data obtained were analyzed using Analysis of Variance, followed by Duncan's Multiple Range Test to determine significant differences. The results revealed that the addition of yellow stripe scad whole powder significantly affected (p<0.05) the physicochemical properties of the yellow stripe scad crackers. Crackers with 15% yellowstripe scad whole powder exhibited the highest protein, lipid, and ash contents of 18.5, 8.97, and 8.97%, respectively. Meanwhile, the addition of 3% yellowstripe scad whole powder produced crackers with the most desirable moisture content, dietary fiber, and texture characteristics of 3.59%, 3.87%, and 1,101 N/cm², respectively.

Keywords: Selar Fish, Flour, Snack, Stunting, Substitution

INTRODUCTION

The yellowstrip scad (*Selaroides leptolepis*), commonly known in Indonesia as the selar fish or yellowtail scad, is characterized by its elongated and laterally compressed body and is widely distributed throughout the Indo-Pacific region (Surajati, 2006; Mustofa and Setyobudiandi, 2020). This species dominates capture fisheries in Indonesian waters, particularly along the northern coast of Java Island and in East Kalimantan, which are part of the national Fisheries Management Areas (WPP) (Widiyastuti et al., 2023). The yellowstripe scad has relatively thin flesh with numerous fine bones; however, its high availability and low market price make it a potential alternative nutrient source. This fish has a complex nutritional composition, including proteins, lipids, and minerals, although its utilization has largely remained limited to traditional dishes such as *pindang* (boiled fish), salted fish, and grilled fish (Sharfina et al., 2014).

Yellowstripe scad can be utilized as a fortificant in cracker production to enhance its nutritional value. Incorporating fish in the form of whole fish powder maximizes nutrient retention and minimizes processing waste. Crackers, which are widely consumed baked products, are versatile and can satisfy diverse consumer preferences (Venkatachalam and Nagarajan, 2017). According to Giannoutsos et al. (2023), crackers are thin, dry, baked products with a crisp texture, typically

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formulated using soft wheat flour as the primary ingredient. They are generally characterized by low levels of sugar, fat, and moisture (Giannoutsos et al., 2023; Venkatachalam and Nagarajan, 2017).) Furthermore, Ahmed and Abozed (2015) classified crackers into three main categories: soda, snacks, and savories.

The fortification of cracker products has emerged as a growing trend, largely because of their long shelf life, ease of accessibility, and relatively low production cost (Hallaç and Altiner, 2017; Giannoutsos et al., 2023; Batista et al., 2019). Currently, cracker fortification efforts commonly utilize ingredients rich in bioactive compounds and essential nutrients, including those derived from cereals and legumes (Giannoutsos et al., 2023). Crackers are among the most popular snack products, with consumer demand increasing steadily every year (Millar et al., 2017). In Indonesia, biscuit consumption was recorded at 21,185 ounces per capita in 2022, rising to 21,215 ounces per capita in 2023, and further increasing to 21,269 ounces per capita by 2024 (Kementan, 2024).

Fortification of crackers using fishery-based products has also been reported by Mutamimah et al. (2024), where the addition of *S. baliensis* / lemuru fish meal significantly affected the moisture, fat, protein, ash, and dietary fiber contents of fish crackers. The inclusion of tuna fish meal significantly enhanced the nutritional quality of the crackers, particularly in terms of protein, fat, moisture, and ash content. Crackers fortified with fish are generally referred to as fish cracker.

Fish crackers can be consumed as dietary food products or alternative nutrient-dense snacks. Given their relatively complex nutritional composition, crackers fortified with yellowstripe scad may also serve as supplementary foods to support stunting prevention, particularly among children in the Balikpapan region. Stunting remains a major public health issue in Indonesia, with alarming prevalence rates. Balikpapan is no exception, as the incidence of stunting has continued to rise, leading to the issuance of Balikpapan Mayor Regulation No. 29 concerning the Guidelines for the Prevention, Screening, and Management of Stunting Cases. The local government has prioritized stunting reduction through nutritional improvement programs. Therefore, this study was conducted to evaluate the nutritional composition (moisture, lipid, dietary fiber, protein, and ash content) and physical quality (hardness) of crackers fortified with yellowstripe scad as a nutrient-rich snack.

MATERIALS AND METHOD

Materials and Properties

The ingredients used for fish cracker production included yellowstripe scad whole powder, high-quality flour (Sania), salt (Dolphin), baking soda (Kupu-Kupu), white crystal sugar (Rose Brand), shrimp stock powder (Alamee), mushroom broth powder (Totole), yeast (Fermipan instant yeast), UHT full-cream plain milk (Indomilk), and room butter (Royal Krane). The materials used in the analysis were filter paper, cotton, hexane, selenium, sulfuric acid, distilled water, sodium hydroxide, boric acid, hydrochloric acid, phosphate buffer, Termamyl enzyme, amyloglucosidase, ethanol, and acetone.

Experimental Design and Data Analysis

The experiment was conducted using a Completely Randomized Design with a single factor and three replicates. The experimental factor was the substitution of yellowstripe scad flour with wheat flour at six levels: 0% (S0), 3% (S3), 6% (S6), 9% (S9), 12% (S12), and 15% (S15). Data were subjected to analysis of variance (ANOVA) using SPSS version 25 and were further evaluated using Duncan's Multiple Range Test (DMRT).

Research Procedure

Preparation of yellowstripe scad whole powder

Yellowstripe scad flour was prepared as described by Mutamimah et al. (2024), with some modifications. The Yellowstripe scad was obtained from the Manggar-Balikpapan Fish Landing Station (TPI) in East Kalimantan. The fish were initially eviscerated to remove the viscera. The cleaned fish were then steamed for 5 min to reduce their moisture content, thereby facilitating the subsequent drying process. The steamed fish were dried using a cabinet dryer at 55 °C for 9 h and milled into powder.

Preparation of fish crackers

The formulation of fish crackers was adapted from the method described by Mutamimah et al. (2024), with modifications. Initially, the ingredients (composite flour (yellowstripe scad flour and high-protein wheat flour), baking soda, sugar, shrimp stock powder, mushroom broth powder, yeast, UHT milk, and margarine (room butter)) were homogenized using a mixer. The formula, based on 150 g of materials, consisted of composite flour and other additional materials.

The composite flour composition based on 59 g flour was obtained by substituting wheat flour with 0.0, 4.5, 9.0, 13.5, 18.0, and 22.5 g of yellowstripe scad whole powder, representing 0, 3, 6, 9, 12, and 15% of the composite flour, respectively. The materials were mixed with baking soda (0.8 g), sugar (0.7 g), shrimp stock powder (0.7 g), mushroom stock powder (2.0 g), yeast (1.0 g), full cream milk (35.25 g), and butter (11.5 g). The dough was then placed in a sealed container and fermented at ambient temperature (30–33 °C) for 60 min. Following fermentation, the dough was shaped into crackers and baked at 145 °C for 5 min. The fish crackers fortified with yellowstripe scad powder are presented in Figure 1.

Figure 1. Fish crackers fortified yellowstripe scad powder

Procedure Analysis

Moisture Content (AOAC, 2012)

The moisture content was determined using a gravimetric method. Empty crucibles were first dried in an oven at 100–105 °C for 30 min, cooled in a desiccator for 30 min, and weighed (C). Approximately 2 g of the sample was placed in a crucible and weighed (D). The crucibles containing the samples were then dried in an oven at 100–105 °C for 24 h. After drying, the samples were cooled in a desiccator and reweighed until a constant weight was obtained (E). The moisture content was calculated using the following equation:

$$MC\ (\%) = \frac{D - E}{D - C} \times 100 \qquad (1)$$

Where:

MC = Moisture content (%)
C = Weight of empty crucible (g)
D = Weight of crucible + sample before drying (g)
E = Weight of crucible + sample after drying (g)

Determination of Fat Content (AOAC, 2012)

The fat content of the fish crackers was determined using the Soxhlet extraction method. Initially, the extraction flask was dried in an oven at 105 °C for 30 min, followed by cooling in a desiccator for 20 min, and weighed (F). Approximately 2 g of the sample was wrapped in filter paper, placed in a thimble, and sealed with cotton wool. The prepared thimble was then inserted into a Soxhlet extractor containing hexane as the solvent. The complete Soxhlet assembly, including the extraction flask, thimble, and condenser, was set up, and extraction was conducted for 5 h. After extraction, the solvent was removed, and the extracted fat was dried in an oven at 105°C for 60 min, cooled in a desiccator, and weighed (G). The fat content of the fish crackers (%) was calculated using the following equation:

$$L(\%\ wb) = \frac{G - F}{S} \times 100$$

$$L\ (\%\ db) = \frac{L\ (\%bb)}{(100 - M)\%bb} \times 100$$

Where:

S = Sample weight (g)
L = Fat content (%)
M = Moisture content fraction of the sample

Determination of Protein Content (AOAC, 2005)

The protein content of the fish crackers was determined using the Kjeldahl method, which consists of three main stages: digestion, distillation, and titration. In the digestion stage, 1 g of the sample was placed in a Kjeldahl flask, followed by the addition of one

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selenium tablet and concentrated H_2SO_4. The mixture was then heated at 400 °C for approximately 60 min until the solution turned green and was subsequently allowed to cool until a solid residue was formed. The residue was quantitatively transferred and diluted with distilled water to a final volume of 100 mL. For distillation, 10 mL of the digested solution was transferred into a distillation flask, followed by the addition of 10 mL 40% NaOH. The released ammonia was distilled and collected in an Erlenmeyer flask containing 10 mL of boric acid solution. Distillation was continued for approximately 15 min until the boric acid solution changed from red to blue. For titration, the distillate was titrated with 0.1028 N HCl until the boric acid solution reverted to its initial red color. The protein content was then calculated based on the nitrogen content using a conversion factor as show in the equation below.

$$\text{Nitrogen (\%)} = \frac{(\text{mL HCl sample} - \text{mL HCl blanko}) \times \text{N HCl} \times 14}{\text{mg sample}} \times 100$$

% Protein = % Nitrogen x conversion factor

Determination of Ash Content (AOAC, 2005)

The ash content of the fish crackers was determined using a gravimetric method. Empty crucibles were dried in an oven at 105 °C for 30 min, cooled in a desiccator for 15 min, and weighed (J). Approximately 5 g of the sample was accurately weighed and transferred into pre-weighed crucibles (K). The crucibles containing the samples were subsequently dried in an oven at 105 °C until no smoke was observed. Thereafter, the samples were ashed in a muffle furnace at 600 °C for 6 h until they were completely converted to ash. Following ashing, the crucibles were cooled in a desiccator for 30 min and reweighed (L). The ash content (%)was calculated using the following equation:

$$\text{Ash \%} = \frac{L - J}{K - J} \times 100$$

Where:

J = Weight of the empty crucible (g)

K = Weight of the crucible with sample before drying (g)

L = Weight of the crucible with sample after ashing (g)

Determination of Dietary Fiber Content in Fish Crackers Using the Enzymatic Gravimetric Method (AOAC, 2012)

Prior to dietary fiber analysis, the fat content of the sample was removed using the Soxhlet extraction method. The filter papers were dried in an oven and weighed to obtain their initial masses. Approximately 0.5 g of the defatted sample was placed in an Erlenmeyer flask, followed by the addition of 25 mL of 0.08 M phosphate buffer at pH 6.0. Subsequently, 0.05 mL of Termamyl enzyme was added, and the mixture was incubated in a shaking water bath at 95 °C for 30 min, followed by cooling. After cooling, 5 mL of 0.275 N NaOH was added to adjust the pH to 7.5, and 0.05 mL of the protease enzyme was introduced. The mixture was then incubated in a shaking water bath at 60 °C for 30 min, and subsequently cooled. The cooled solution was adjusted to pH 4.0–4.5 using 5 mL of 0.325 N HCl, and 0.15 mL of amyloglucosidase was added. The mixture was then incubated at 60 °C for 30 min in a shaking water bath. The final hydrolysate was filtered through pre-weighed Whatman No. 42 filter paper using a vacuum filtration system (B1).

Insoluble Dietary Fiber (IDF)

The residue retained on the filter paper was sequentially washed with 2 × 15 mL of 78% ethanol, 2 × 15 mL of 95% ethanol, and 2 × 15 mL of acetone. The filter paper containing the residue was then transferred to an empty aluminum dish, dried at 105°C for 12 h to a constant weight, cooled in a desiccator, and weighed (B2). The residue was subsequently analyzed for protein and ash content for correction purposes.

Soluble Dietary Fiber (SDF)

The filtrate was adjusted to a total volume of 70 mL with distilled water, followed by the addition of 279 mL of preheated 95% ethanol at 60 °C. The mixture was incubated for 60 min to precipitate SDF and then filtered through filter paper. The residue was sequentially washed with 2 × 15 mL of 78% ethanol, 2 × 10 mL of 95% ethanol, and 2 × 10 mL acetone. The filter paper containing the residue was transferred to an empty aluminum dish, dried at 105°C for 12 h, cooled in a desiccator, and weighed (B2). The protein and ash contents of the

residue were subsequently analyzed for correction. The dietary fiber content of the fish crackers on a wet weight basis was calculated using the following formula:

$$\text{IDF (\%wb)} = \frac{\text{B2-B1}}{\text{sampel (g)}} \text{ - \% ash residu-\%protein residu (g)}$$

$$\text{SDF (\%wb)} = \frac{\text{B2-B1}}{\text{sample weight s (g)}} \text{ - \% ash residue - \% protein residue (g)}$$

$$\text{TDF (\%wb)} = \text{IDF} + \text{SDF}$$

The dietary fiber content on a dry weight basis (db) was calculated to account for the moisture content of the samples using the following equation:

$$\text{IDF (\%db)} = \frac{\text{\%IDF}}{\text{100\%-moisture content \%}} \text{x100}$$

$$\text{SDF (\%db)} = \frac{\text{\%SDF}}{\text{100\%-moisture content \%}} \text{x100}$$

$$\text{TDF (\%db)} = \frac{\text{\%TDF}}{\text{100\%-moisture content \%}} \text{x100}$$

Texture (Hardness)

The hardness was determined using a penetration test with a Texture Analyzer TA-XT2. The instrument was set with the following parameters: pre-test speed of 2.0 mm/s, post-test speed of 5.0 mm/s, penetration distance of 5.0 mm, test duration of 5.0 s, and an automatic trigger system with a trigger force of 10 g. Cracker samples were placed on a support rig and tested using a stainless-steel ball probe with a diameter of 0.25 in. (p/0.25 s). This setup was used to evaluate the product fracturability through penetration testing. The texture parameter measured in this study was hardness, expressed in N/cm² (Huda and Ikhlas, 2009.)

RESULT AND DISCUSSION

The macronutrient components of yellowstripe scad crackers were determined to evaluate their nutritional value as a potential functional food for stunting prevention. The physical quality of the crackers was further assessed using instrumental texture analysis. Texture is a critical parameter for consumer acceptance, as crackers are typically expected to possess both hardness and crispness. The results of the macronutrient and physical property analyses, including protein, moisture, fat, dietary fiber content, and texture, are presented in Table 1, and in a form of diagram in Figure 2 to show the profile characteristic changes more clearly.

Moisture Content

Moisture content is a critical parameter in crackers, as it directly affects product crispness, texture, and shelf life (Venkatachalam and Nagarajan, 2017). According to Yanti et al. (2023), the moisture content is closely associated with the shelf life of food products, as it affects their physical, chemical, and microbiological properties and enzymatic activity.

The addition of 3% yellowstripe scad (*Selaroides leptoletis*) flour compared to the control did not significantly affect the moisture content of fish crackers ($p < 0.05$). Comparable results were observed among 3, 6, and 9% yellowstripe scad whole powder additions, whereas significant differences emerged at 12 and 15% flour incorporation. The results of the moisture content analysis of yellowstripe scad -based fish crackers are presented in Figure 2a, Table 1.

Table 1. Chemical and physical composition of yellowstripe scad crackers

Composite flour (% yellowstripe scad flour)	Moisture content (%)	Protein (%)	Lipid (%)	Ash (%)	Dietary Fiber (%)	Texture (N/cm²)
0 (S0)	3.52 [a]	8.15 [a]	4.12 [a]	1.56 [a]	5.65 [a]	933 [a]
3 (S3)	3.59 [ab]	10.29 [b]	8.09 [b]	3.92 [b]	3.87 [b]	1,101 [b]
6 (S6)	3.68 [b]	12.41 [c]	10.67 [c]	4.01 [b]	3.01 [b]	1,281 [c]
9 (S9)	3.78 [bc]	14.55 [d]	13.02 [d]	5.87 [c]	2.32 [c]	1,374 [d]
12 (S12)	3.86 [c]	16.76 [e]	15.05 [e]	6.14 [c]	1.08 [d]	1,432 [e]
15 (S15)	3.97 [c]	18.54 [f]	17.31 [f]	8.97 [d]	0.98 [d]	1,609 [f]

Note: Data (mean±SD) were calculated from five replicates. The data were analyzed by ANOVA. Data within the same column followed by different letters showed significantly different (Tukey test, $p<0.05$).

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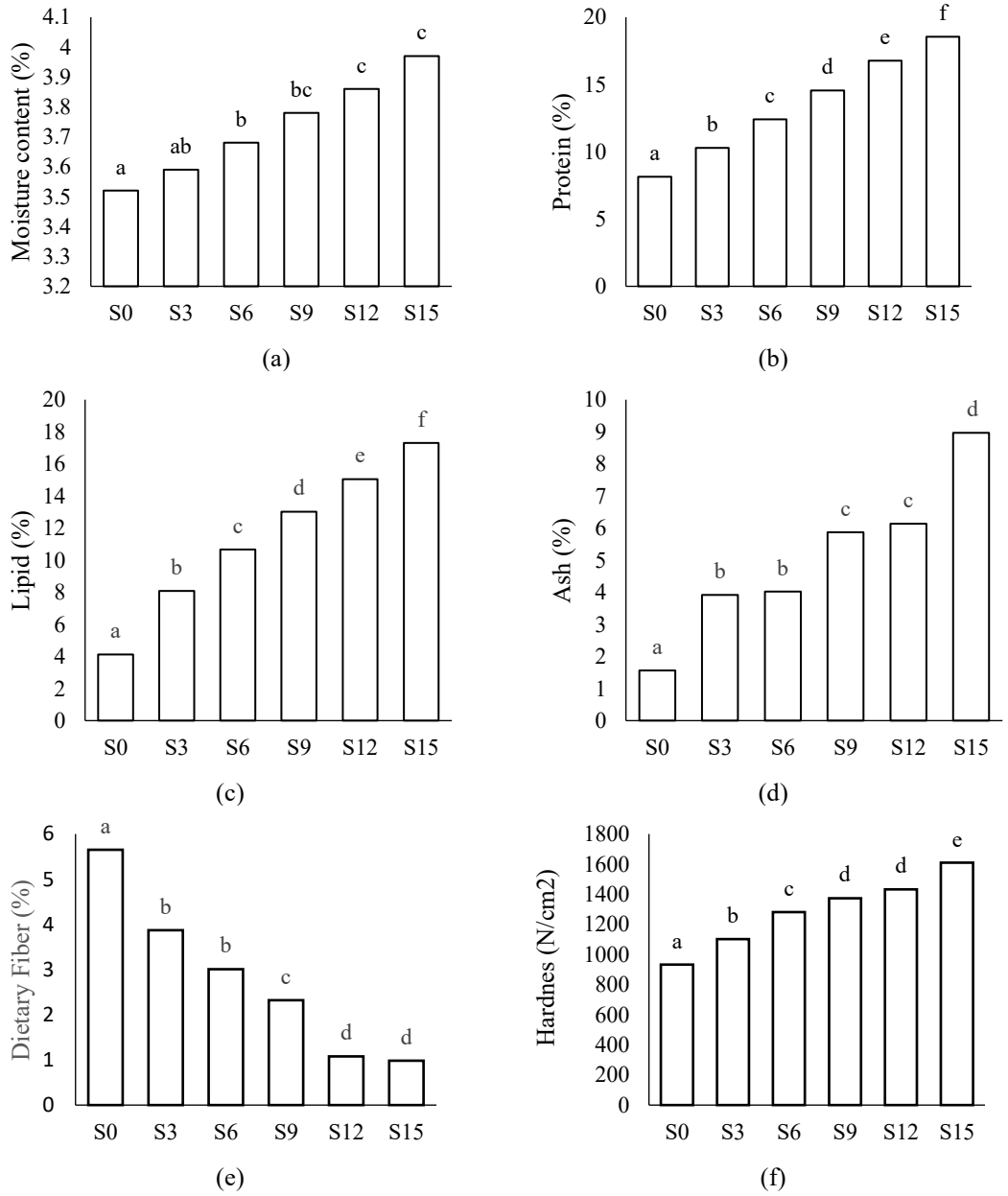

Figure 2. Effects of yellowstripe scad powder on chemical properties and texture of yellowstripe crackers. S0 – S6 were referred to Table 1.

The moisture content of fish crackers ranged from 3.52% to 3.97%, with the highest value observed in samples containing 15% yellowstripe scad whole powder (S5) at 3.97% and the lowest in the control sample without fish addition (S0) at 3.52%. An increase in the concentration of yellowstripe scad whole powder corresponded to a gradual increase in the moisture content of the crackers. This trend is consistent with the findings of Ernisti (2018), who reported that higher amounts of Siamese catfish flour led to an increase in the moisture content of crackers. Similarly, Breemer et al. (2023) reported that the addition of skipjack tuna (*Katsuwonus pelamis*) flour was positively correlated with a higher moisture content in tortilla chips.

Protein Content

The protein content of the yellowstripe scad whole powder crackers ranged from 8.15% to 18.54%. Analysis of variance

(ANOVA) demonstrated that the addition of yellowstripe scad (*Selaroides leptoletis*) flour significantly affected the protein content of the crackers ($p < 0.005$). The highest protein content was observed in the formulation containing 15% fish flour (S5), whereas the lowest was found in the control sample without fish flour (S0). Increasing the concentration of yellowstripe scad whole powder correspondingly increased the protein content of the fish crackers. The protein content of the yellowstripe scad crackers is shown in Figure 2b and Table 1b**.**

Similar findings were reported by Mutamimah et al. (2024), who showed that crackers substituted with sardine head flour at concentrations of 5–20% exhibited protein content ranging from 8.03% to 14.23%. Similarly,other studies have demonstrated that the incorporation of tuna flour increases the protein content of crackers. Comparable results were also observed by Nawaz et al. (2019), who found that the addition of whole fish powder (WFP) at concentrations of 5–15% significantly enhanced the protein content of crackers, with values ranging from 14.23% to 19.56%.

According to Sinaga et al. (2018), fresh yellowstripe scad (*Selaroides leptolepis*) contains a relatively high protein content of approximately 18.8%, which decreases to approximately 5.3% when it is processed into a fish hydrolysate. This characteristic explains the elevated protein levels in crackers fortified with yellowstripe scad whole powder compared to those enriched with other fish flours at equivalent substitution levels. The higher protein content observed in this study can also be attributed to the use of whole fish flour prepared from the entire body of yellowstripe scad, which is consistent with the findings of Nawaz et al. (2019).

Lipid Content

Lipid content is important in food products, as it affects both their nutritional value and shelf life. In general, higher lipid levels are associated with a reduced shelf life owing to increased susceptibility to lipid oxidation. The results of the flipid content analysis of yellowstripe scad crackers are shown in Figure 2c and Table 1.

The lipid content of the fish crackers ranged from 4.12% to 17.31%. Analysis of variance revealed that the incorporation of yellowstripe scad (*Selaroides leptoletis*) flour significantly affected the fat content of the crackers ($p < 0.005$). The highest lipid content was recorded in samples containing 15% fish flour (S5), whereas the lowest was observed in the control sample without fish flour (S0). A progressive increase in lipid content was evident with higher substitution levels of yellowstripe scad whole powder. These results are consistent with those of Mutamimah et al. (2024), who reported that the inclusion of Bali sardine flour at levels of 5–20% increased the lipid content of fish crackers, ranging from 11.43% to 16.85%.

Ash Content

Ash represents the micromolecular chemical component of food, derived from the inorganic residue that remains after the complete combustion of organic matter under controlled temperature and pressure. The ash content of a food product reflects its mineral composition, which generally consists of both organic and inorganic salts (Pangestuti and Darmawan, 2021). The ash content of the yellowstripe scad crackers obtained in this study is shown in Figure 2d and Table 2.

The ash content of yellowstripe scad crackers ranged from 1.56% to 8.97%. The addition of yellowstripe scad (*Selaroides leptoletis*) flour significantly affected the ash content of the crackers ($p < 0.005$). The highest ash content was observed in samples with 15% fish flour (S5), whereas the lowest was observed in the control sample without fish flour (S0). Increasing the concentration of yellowstripe scad whole powder resulted in a proportional increase in ash content, consistent with the findings of Mutamimah et al. (2024), who reported that the mineral content of Bali sardine-based fish crackers increased with higher levels of sardine head flour.

The addition of tuna flour significantly elevated the ash content of the fish crackers. The higher ash content in yellowstripe scad crackers observed in the present study can also be attributed to the use of whole yellowstripe scad powder prepared from the entire body of yellowstripe scad. This finding

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aligns with that of Nawaz et al. (2019), who demonstrated that incorporating whole fish powder into cracker production significantly increased ash content, primarily due to the high levels of hydroxyapatite and collagen derived from fish bones.

Dietary Fiber

Dietary fiber is an essential component of food that plays a critical role in supporting human health, particularly by enhancing the gastrointestinal function. However, excessive dietary fiber negatively affected the sensory quality of yellowstripe scad fish crackers (Figure 2e and Table 2).

The dietary fiber content of yellowstripe scad crackers ranged from 0.98% to 5.65%. The addition of yellowstripe scad whole powder (*Selaroides leptoletis*) significantly affected the dietary fiber content of the crackers ($p < 0.005$). The highest fiber content was observed in the control sample without fish flour (S0), while the lowest was observed in crackers with 15% fish flour (S5). Increasing the concentration of yellowstripe scad whole powder resulted in a progressive decrease in dietary fiber content.

This finding is consistent with that of Mutamimah et al. (2024), who reported that higher levels of fish flour increased fat content, which in turn reduced fiber levels. Similarly, Nawaz et al. (2019) demonstrated that the incorporation of whole fish powder, characterized by low starch and fiber content, reduced both carbohydrate and fiber content, accompanied by an increase in fat content. To enhance the dietary fiber content of fish crackers, plant-based ingredients rich in fiber, such as mung bean flour, should be incorporated. In addition to improving the fiber content, the inclusion of mung bean flour mitigated the characteristic "fishy" odor in fish crackers.

Texture (Hardness)

Texture is a critical quality attribute of crackers, with hardness and fracturability serving as key parameters that influence consumer perceptions of crispness and crunchiness. These properties are highly desirable in cracker-type products (Batista et al., 2019; Millar et al., 2017; Yilmaz and Karaman, 2017). According to Tunick et al., (2013), texture is among the most important quality attributes of crackers, as consumers strongly associate these products with a crisp and crunchy eating experience. The results of the hardness analysis of the yellowstripe scad crackers are presented in Figure 2f and Table 2.

The addition of of yellowstripe scad (*Selaroides leptoletis*) flour had a significant effect on the texture of fish crackers ($p < 0.005$). Increasing the concentration of yellowstripe scad whole powder resulted in greater hardness of the crackers. The highest hardness was observed in samples with 15% fish flour (S15), whereas the lowest was observed in samples with 3% fish flour (S3).

However, contrasting findings have been reported in other studies. Breemer et al. (2023) found that increasing the amount of skipjack tuna (*Katsuwonus pelamis*) flour decreased the hardness of tortilla chips, as higher flour concentrations were associated with increased product moisture. Similarly, Giannoutsos et al. (2023) observed that higher levels of substitution reduced the hardness and fracturability of crackers. This effect was attributed to the reduced availability of wheat flour, which lowers the content of gliadin and glutenin proteins essential for CO_2 retention during dough fermentation, thereby producing a more elastic and expanded dough (Giannoutsos et al., 2023; Nammakuna et al., 2016)

Moreover, dietary fiber has been shown to negatively affects product crispness by impairing dough rheology and uniformity (Venkatachalam and Nagarajan, 2017). Bolek (2020) further reported that a higher fiber content increases the water absorption capacity, which can reduce the texture quality of the cracker. In the present study, however, increasing the levels of yellowstripe scad whole powder was associated with a decrease in the dietary fiber content, which may partly explain the observed increase in cracker hardness.

CONCLUSION

The results of this study indicate that the addition of yellowstripe scad whole powder had a significant effect ($p < 0.005$) on the physicochemical properties of yellowstripe scad crackers. Crackers with

15% yellowstripe scad whole powder exhibited the highest protein (18,5%), lipid (8,97)%), and ash content (8,97%), while the addition of 3% yellowstripe scad whole powder produced crackers with the most desirable moisture content (3,59%), dietary fiber (3,87%), and texture (1,101 N/cm²) characteristics.

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COMPARATIVE EFFECTIVENESS OF ACID AND WATER DEGUMMING METHODS IN CRUDE PALM OIL (CPO) PRETREATMENT

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ABSTRACT

Crude Palm Oil (CPO) is the primary raw material in the cooking oil industry; it must be refined to improve its quality and stability. Degumming is a critical pretreatment step in CPO refining, as it removes phospholipids that can affect the oil quality. This study aimed to evaluate the effects of two degumming methods, acid degumming and water degumming, on various quality parameters of CPO, including moisture content, free fatty acids (FFA), tocopherols, beta-carotene, and peroxide levels. The results showed that both degumming methods significantly reduced the FFA content and peroxide number. Acid and water degumming were effective in lowering the FFA content and removing impurities from crude palm oil (CPO). They reduced the FFA level by 79.8-80.7% and by 15.9-17.3% for the peroxide number. Although reductions in α-tocopherol were observed, they remained acceptable and did not affect nutritional value.

Keywords: crude palm oil, acid degumming, water degumming, oil quality, oxidative stability

INTRODUCTION

Crude palm oil (CPO) is one of Indonesia's top commodities and is vital to both the food and non-food sectors. Due to its high triglyceride content, CPO is a major raw material used to produce biodiesel, margarine, cooking oil, and soap (Helwani et al., 2021). However, CPO must undergo several refining procedures to eliminate contaminants that could lower the stability and quality of the final product before it can be utilized extensively (Setyawardhani and Satiawardana, 2025).

CPO must undergo chemical and physical purification procedures, such as degumming, bleaching, and deodorization, to lower the water content, FFA, free fats, phospholipids, and other contaminants to make it safe and stable (Hidayati et al., 2023; Alhaji et al., 2024). An extra washing step prior to purification helps suppress pollutants like 3-MCPDE to meet European Union requirements during stringent regulations (Hidayati et al., 2023). The goal of the purification process is to preserve the color, odor, thermal stability, and shelf life of the final product. The difficulty lies in preventing the production of hazardous compounds during the processing.

Degumming, the removal of phospholipid molecules from oil, is a crucial stage in the refining process. Polar, emulsifying substances called phospholipids can make oil unstable when heated or stored, and phospholipids are classified as either hydratable or non-hydratable in CPO (Bourtsala and Galanopoulou, 2019). To successfully separate each of these types from the oil phase, specific handling is required.

The elimination of phospholipids, which are polar emulsifying substances that may lead to instability during processing or storage, is a crucial stage in oil purification, particularly degumming. Hydratable phospholipids (HP), which are readily separated with water, and non-hydratable phospholipids (NHP), which attach firmly and require acidic or enzymatic treatment for successful separation, are the two forms of phospholipids found in oil (Pan et al., 2024). For instance, adding acids such as citric or phosphoric acid during the degumming process helps change NHP into a form that is

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easier to separate by causing flocculation, followed by centrifugation.

Acid and water degumming are two common degumming techniques used in the industry. While acid degumming entails adding an acid, usually phosphoric acid (H_3PO_4), to change non-hydratable phospholipids into a state that can be eliminated by hydration, water degumming employs warm water to remove hydratable phospholipids, such as lecithin. These two techniques are frequently coupled to improve oil quality, as they differ in their ability to lower phospholipid and oil pollutant levels (Matthäus and Pudel, 2014).

Moisture content, free fatty acids (FFA), carotenoids, tocopherols, and peroxide value are a few of the quality indicators of palm oil that are greatly affected by the degumming process. The oxidative stability of the oil is enhanced by the controlled application of acid degumming, which successfully lowers the FFA and peroxide values without reducing minor components such as tocopherols. The moisture level may be regulated to ±0.25%, and the carotenoid concentration remains at approximately 381.350 ppm under ideal conditions (80 °C) (Nidzam et al., 2022; Hidayat et al., 2024; Manurung, 2023). To ensure that degummed oil satisfies both national and international requirements, it is crucial to evaluate these quality factors.

This study aimed to evaluate the effects of acid and water degumming techniques on the physicochemical characteristics of CPO, including moisture content, free fatty acids (FFA), β-carotene, tocopherol, and peroxide value. The efficacy of each technique in creating a productive and standard-compliant CPO purification process is anticipated to be determined by comparing acid degumming with water degumming.

MATERIALS AND METHODE

Materials and Tools

The main material used was CPO sourced from commercial palm oil processing companies in East Kalimantan. The samples, including freshly pressed CPO, were collected and stored for processing to evaluate the effect of storage on degumming efficacy. The chemicals used were analytical grade (PA) acetic acid, NaOH (Merck, Germany), phenolphthalein indicator, starch indicator, alcohol (One Med, Indonesia), and $Na_2S_2O_3$ (Merck, Germany).

Method

Degumming phosphoric acid accounts for 85% of the acid degumming process. After heating the CPO to 60 °C, 0.09% phosphoric acid was added. This implies that for every liter of solution (CPO + phosphoric acid), there are 0.09 liters of 85% phosphoric acid. The CPO solution was agitated and cleaned until the pH was neutral. The CPO was heated at 60 °C and sprayed with water (washed) in a 1:1 ratio for water degumming.

Determination of FFA, Peroxide Value and Water Content

This experiment tested the peroxide value using the AOAC (Association of Analytical Communities) titration method, free fatty acid using acidic base titration, and water content using the gravimetric method.

Determination of Alpha Tocopherol

The oil samples were dissolved in ethanol, and the absorbance was measured at 221 nm. The tocopherol content of the oil samples was expressed as mg α-tocopherol/kg oil (ppm).

Determination of Carotenoids

Carotenoids were determined according to the PORIM test method (PORIM, 1995). Oil samples were dissolved in hexane, and the absorbance was measured at 446 nm. The carotenoid content of the oil samples was expressed as mg β-carotene/kg oil (ppm).

Experimental Design and Data Analysis

This experiment was a single-factor experiment (product type), namely CPO, acid-degummed oil, and water-degummed oil. The treatments were replicated five times for each treatment. Data were analyzed using analysis of variance (ANOVA), followed by Tukey's test at $\alpha = 5\%$.

RESULTS AND DISCUSSION

Pre-treatment of CPO by degumming showed significant ($p<0.05$) positive effects on reducing the FFA and peroxide numbers.

However, it also significantly (p<0.05) reduced the α-tocopherol content. The β-carotene and water content in the degummed oil remained as CPO. Neither degumming method significantly affected any of the chemical characteristics observed (Table 1).

Table 1. Chemicals characteristics of CPO and degummed oil

Product	Water content (%)	FFA (%) as palmitate	β-Carotene (ppm)	α-Tocopherol (ppm)	Peroxide Number (meq. O_2/kg)
CPO	0.24 ± 0.05	1.14 ± 0.09a	53.59 ± 1.19	108.55 ± 0.99a	2.31 ± 0.11a
Acid degumming	0.23 ± 0.02	0.23 ± 0.02b	52.10 ± 1.27	99.53 ± 0.87b	1.95 ± 0.09b
Water degumming	0.22 ± 0.03	0.22 ± 0.04b	53.21 ± 2.88	100.55 ± 1.34b	1.91 ± 0.09b

Note: Data (mean ± SD) were calculated from five replicates each. The data were analyzed using ANOVA. The data within the same column followed by different letters indicate significant differences (Tukey's test, p<0.05).

Water Content and FFA (Free Fatty Acid)

Moisture content is a critical parameter for determining oil quality. A high moisture level indicates the presence of significant water in the oil, which accelerates hydrolytic reactions and promotes the breakdown of triglycerides.

The water content of CPO after treatment via either acid or water degumming did not differ significantly (Table 1). This is because the water content in the oil reflects the accuracy of oil–water separation through the decanting funnel during the washing step.

Acid degumming, which uses phosphoric acid (H_3PO_4) and warm water (~60 °C), is a typical degumming method used in the manufacturing of palm frying oils. Phosphoric acid and warm water are used to remove non-hydratable phosphatides. Phosphatidylethanolamine (PE) is the main constituent of non-hydrated phosphatides. Phosphoric acid transforms it into a hydratable form, whereas hydratable phosphatides, such as phosphatidylcholine (lecithin), can be readily extracted from the oil using cold water (approximately 40 °C). This causes the phospholipids to become lipophobic instead of lipophilic, making them easier to separate from the oil (Dijkstra and Opstal, 1987; Lamas et al., 2016; McClements and Jafari, 2018).

The quality of the oil is lowered by residual water or water that is left in the oil after degumming. The Indonesian CPO standard (SNI 01-2901-2006) (BSN, 2006) requires a maximum moisture and impurity concentration of 0.5 %. The water content of the oil in the current study remained between 0.22% and 0.24%, which is the maximum limit. According to recent research, moisture levels in palm oil following degumming and refining procedures usually fall between 0.24 and 0.25 percent (Teye et al., 2023; Ominyi and Isaac, 2024). The phosphorus and residual phospholipid content in palm oil are greatly affected by degumming process parameters, such as phosphoric acid dosage, temperature, and time (Nidzam et al., 2022).

Triglycerides, which constitute the majority of oils, are hydrolyzed and oxidized to produce free fatty acids (FFA). Because the reaction converts neutral oil into glycerol and FFA, hydrolysis lowers the oil quality. In particular, the FFA produced have a rotten taste and smell (Ketaren, 2008).

Triglycerides, which constitute the majority of oils, are hydrolyzed and oxidized to produce free fatty acids (FFA). Because the reaction converts neutral oil into glycerol and FFA, hydrolysis lowers the oil quality. Particularly in short-chain fatty acids, the FFA that are produced have a rotten taste and smell (Ketaren, 2008). This illustrates that while there is no discernible difference in the FFA content between the acid and water degumming processes, there is a discernible difference when compared to crude palm oil (CPO) without degumming, where the degumming treatment significantly reduced the FFA content. This suggests that some FFA dissolved in the aqueous phase during the degumming process due to the use of water. The addition of water to the degumming process can hasten the breakdown of oil through enzyme activity. By limiting the time that water and oil come into contact and speeding up phase separation, proper handling throughout the degumming process can stop or lessen hydrolysis. The removal of gums coincided with a decrease in the FFA content in CPO that underwent aqueous degumming. Degumming CPO to remove phospholipids

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resulted in a slight decrease in FFA in the oil, from 1.14% in untreated CPO to 0.61% after acid degumming and 0.70% after water treatment.

The degumming process precipitates or eliminates non-triglyceride materials, such as gums, which are often linked to lipase enzymes and water molecules. Triglycerides are broken down into FFA by lipase enzymes. Degumming gums and contaminants inhibit lipase action, significantly lowering the rate of fresh FFA synthesis (Shahidi & Zhong, 2010). This explains why a significantly more noticeable decrease in FFA content is observed following degumming compared to untreated CPO. Sufficient drying to eliminate excess water used in the degumming stage before further processing is another reason for the drop in FFA. By reducing the time that oil and water are in contact and promoting quick separation, proper handling of the washing stage can prevent or lessen hydrolysis.

Beta Carotene and Tocopherol

The β-carotene (ppm) and tocopherol content of CPO, acid degumming, and water degumming are presented in Table 1. The acid degumming approach with phosphoric acid at 60 °C reduced the β-carotene content from 53.59 ppm to 52.10 ppm (3%), whereas the water degumming method reduced it to 53.21 ppm (1%). Nevertheless, the difference in the decrease between the two approaches was not statistically significant according to the analysis of variance. However, the mean values do reveal a decrease, which is consistent with earlier research showing that the degumming process entrains oil fractions containing β-carotene during separation, in addition to removing water (Sulihatimarsyila et al., 2020; Oey et al., 2020). Degumming and more thorough refining procedures result in significant reductions in carotenoid content, according to recent studies (Widigdo et al., 2023; Hidayat et al., 2025). Further losses of β-carotene have been reported during the distillation and purification of red palm oil, suggesting that the carotenoid fraction may be partially "absorbed," degraded, or removed during these purification steps (Jusman et al., 2023).

Furthermore, the initial α-tocopherol concentration of 108.55 ppm decreased to 99.53 ppm (−8%) and 100.55 ppm (− 7 %) under the acid and water degumming methods, respectively. Analysis of variance indicated that this reduction was statistically significant. The decline in both β-carotene and α-tocopherol levels is influenced by phosphoric acid and the water used during the washing stage (Tucker and Townsend, 2005; Hambly et al. 2021). Washing oil with water can significantly reduce tocopherol content. These results suggest that tocopherol is more readily lost or dissolved during washing than β-carotene.

Peroxide Number (meq O_2/kg)

When unsaturated fatty acids react with oxygen, peroxides are formed (Gunstone, 1984). A higher peroxide value (PV) indicates greater oil oxidation (Yin and Porter, 2011). The peroxide value of an oil indicates the early oxidation stage of fats or oils (Shahidi and Zhong, 2010). Therefore, PV is the main signal of oil deterioration, particularly in the early stages prior to the formation of secondary chemicals such as aldehydes and ketones, which provide rancid flavor and odor (Gotoh and Wada, 2006; Shahidi and Zhong, 2010). Overall, the PV measured in the treatment remained below the maximum peroxide value of 10 meq O_2/kg stipulated by the Indonesian national standard (SNI 3741:2013) for cooking oil (BSN, 2013). The peroxide value dropped after the degumming treatment, from 2.31 meq O_2/kg to 1.95-1.91 meq O_2/kg, although the decline was not statistically significant. Certain contaminants, including physical impurities, phospholipids, and free fatty acids, can be eliminated by washing oil with hot water during the degumming process. However, because the peroxide molecules are already dissolved in the oil phase and are chemically stable, hot-water washing is not very effective in achieving a considerable reduction in the peroxide value.

CONCLUSION

Acid and water degumming effectively lowered free fatty acids (FFA) and removed impurities from crude palm oil (CPO). Both can reduce FFA to the level of 79.8-80.7% and 15,6-17.3% for peroxide number. Although reductions in α-tocopherol were observed,

they remained acceptable and did not affect nutritional value.

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ANTIMICROBIAL ACTIVITY OF KOMBUCHA DRINK MADE OF TORCH GINGER (*Etlingera elatior*) FLOWERS TOWARDS PATHOGENS

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ABSTRACT

Kombucha is a functional drink with benefits for human health, including antimicrobial activity. It could be made from torch ginger, an Indonesian tropical plant that is known as a vegetable and ornamental plant and has antimicrobial activity against pathogens. This study aimed to determine the effect of fermentation time and sugar content of kombucha made from torch ginger flowers on antimicrobial activity against pathogens. The research was conducted with two treatments: 20, 25, and 30% (w/v) sugar addition and 7, 14, and 21 days of fermentation. The antimicrobial activity of the kombucha drink made from 12% torch ginger was evaluated using the well diffusion method. The test pathogens were Escherichia coli ATCC 11775, *Bacillus cereus* ATCC 11778, Staphylococcus aureus ATCC 33591, and *Salmonella typhi* ATCC 14028. The results showed that torch ginger kombucha with 20% sugar and fermented for 14 days significantly inhibited Staphylococcus aureus ATCC 33591 and Bacillus cereus ATCC 11778. The Minimum Inhibitory Concentration (MIC) against both pathogens was observed in 20% kombucha. Therefore, kombucha made from torch ginger has the potential to be an antimicrobial against pathogens.

Keywords: Antimicrobial; Functional drink; fermentation time; Well diffusion Methods

INTRODUCTION

Fermentation is one of the oldest food-preservation techniques. Kombucha is a traditional fermented beverage that has been consumed since 220 BC (Wang *et al*., 2022). This beverage is fermented from a sugary tea drink through the metabolic activity of a Symbiotic Culture of Bacteria and Yeast (SCOBY), primarily characterized by a synergistic relationship between acetic acid bacteria (AAB), including *Komagataeibacter*, *Gluconobacter*, and *Acetobacter,* and lactic acid bacteria (LAB), specifically *Lactobacillus* and *Lactococcus*.

Kombucha is recognized for its potential bioactivity to enhance human health. The widespread consumption of these beverages is due to their bioactive compounds, including various essential vitamins (B1, B2, B3, B6, B12, B15, and C), organic acids such as glucuronic acid and acetic acid, polyphenols with potential antioxidant activities (Cholidah et al., 2020), essential amino acids, folic acid, and various enzymes, which can be beneficial to human health (Khamidah and Antarlina, 2020).

Furthermore, investigations have explored the utilization of soursop leaves (*Annona muricata*), rhizomes such as ginger (*Zingiber officinale*), and non-traditional bases such as bamboo shoot process water (*rebu*). Kombucha fermentation can be combined with various plants, such as rosella, butterfly pea, and torch ginger (Indonesian: *kecombrang*) flowers. Some studies have explored the combination of tea leaves with soursop leaves, ginger, and corn silk tea. It was found that rosella kombucha containing acetic acid, which is used as an antibacterial agent, had a significant effect on the antibacterial activity of *Escherichia coli* (Cholidah et al., 2020). The combination of kombucha with butterfly pea flowers has anthocyanin content that showed potential inhibition of the growth of bacteria *Salmonella typhi* and *Escherichia coli* (Sefrina, 2021).

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Torch ginger (*Etlingera elatior*), a member of the *Zingiberaceae* family, contains a wide range of bioactive compounds, including phenolics, flavonoids, alkaloids, saponins, and terpenoids, which contribute to its antibacterial and antioxidant activities (Rasyadi et al., 2022). The beneficial effects of kombucha are associated with the presence of bioactive compounds, including D-saccharic acid-1,4-lactone, gluconic acid, glucuronic acid, tea polyphenols, vitamins, amino acids, various micronutrients, and antibiotics. The antioxidant and antimicrobial activities of kombucha are mainly attributed to the presence of these bioactive compounds (Ojo and de Smidt, 2023).

Fermentation into kombucha could enhance the antimicrobial activity compared to non-fermented torch ginger, due to the increased production of organic acids and the formation of other bioactive metabolites during the fermentation process (Mizuta *et al*., 2020. Kombucha drink with torch ginger extract was able to inhibit *Escherichia coli* and *Staphylococcus aureus* at sugar concentrations of 20%, 30%, 40%, and 50% with a fermentation duration of 12 days (Fadhilah et al., 2023). Kombucha drinks made from torch ginger extract also showed antibacterial activity against *Escherichia coli*, *Salmonella thypimurium*, and *Staphylococcus aureus* after a fermentation period of 9 days and the addition of 2.5% granulated sugar. (Sintyadewi et al., 2023).

In this study, kombucha was combined with torch ginger extract and used as a fermented beverage. This study focused on the antimicrobial potential of kombucha torch ginger extract in inhibiting *Escherichia coli* ATCC 11775, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 33591, and *Salmonella thypi* ATCC 14028, considering the duration of kombucha fermentation and the addition of different palm sugar concentrations.

MATERIALS AND METHOD

Material

The materials used in this study were torch ginger (*Etlingera elatior*) flowers obtained from the Beluluq Lingau market on Jalan PM Noor, Samarinda City, SCOBY obtained from an online shop, Tuana Tuha palm sugar under the Gulaku Premium Sugar brand obtained from local shop in Samarinda.

Experimental Design and Sample Preparation

The experimental design used in this study was a completely randomized design consisting of two factors, each with three levels of treatment. The first factor was fermentation time with three treatment levels: 7, 14, and 21 d. The second factor was the addition of sugar at different concentrations, which had three treatment levels, namely 20, 25, and 30%, as well as positive and negative controls with three replicates.

In the first stage of the research, the results were in the form of inhibition zones resulting from antimicrobial activity tests based on different sugar concentrations and fermentation periods. The best treatment was indicated by the diameter of the inhibition zone formed around the well. The diameter of the inhibition zone was measured using a caliper in mm.

Data Analysis

The data obtained in this study were analyzed using Analysis of Variance continued by Tukey test using SigmaPlot 14.0 data-processing application. The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests are presented in a descriptive form.

Procedure for Making Torch ginger Extract

The first step was to cut the flowers from the stems and wash them with clean water. After washing, the samples were blended to smoothen the mixture. The next step was to weigh 250 g of torch ginger flowers in 2 liters of water and boil them for 30 min on a hotplate at the maximum temperature. The final step was to filter the torch ginger flowers from the torch ginger flower extract.

Procedure for Making Kombucha Drink from Torch ginger Extract

The production of torch ginger extract kombucha refers to the procedure (Abdilah et al., 2022). Two liters of boiled torch ginger extract was divided into three parts for nine

jars with the addition of sugar according to the treatment, namely 20, 25, and 30% (b/v) for one repetition. Next, the boiled torch ginger extract was cooled to ± 25 °C, and a kombucha starter, namely a 9 cm diameter SCOBY, was added to each treatment at 25% (v/v). The glass jars were then tightly sealed using their lids and fermented according to the treatment for 7, 14, and 21 d at room temperature away from direct sunlight.

Analysis Procedure

Preparation of Test Bacterial Culture (Ningtyas, 2015)

The test microorganisms were pure cultures of *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATCC 14028. Before cultivation, each bacterial culture was refreshed by reinoculation in a test tube containing 10 mL of Nutrient Broth (NB) medium and incubated in an incubator at 37 °C for 24 h.

Antimicrobial Activity Test

The antimicrobial activity of torch ginger extract kombucha was tested using the well diffusion method, as described by Nikmah (2020), with slight modifications. The inoculation process was carried out by applying the bacterial suspension to the surface of the NA medium in a dish using a sterile cotton swab and then left for 5 min to allow the suspension to soak evenly. Next, five wells with a diameter of 6 mm were created in each dish. The torch ginger extract kombucha with various sugar concentrations (20, 25, and 30%) and various fermentation times (7, 14, and 21 days), and the positive control of amoxicillin 500 mg (for bacteria) as the negative control, were each poured into the wells at 40 µL (0.04 mL). All Petri dishes containing the test samples were incubated for 24 h at 37°C.

$$\text{Inhibition Zone Diameter (mm)} = \frac{D1+D2+D3}{3} - \chi$$

Description:
D1 = Vertical Diameter, D2 = Horizontal Diameter, D3 = Diagonal Diameter, χ = 6 mm Well Diameter

Determination of Minimum Inhibitory Concentration

This Minimum Inhibitory Concentration (MIC) assay used the well diffusion method, which refers to research conducted by Rahman et al. (2022). The kombucha drink from torch ginger after 14 d of fermentation and with 20% pal sugar was diluted with sterile aquadest to make solutions with varying concentrations: original (0.5 mL kombucha), 80% (0.4 mL kombucha + 0.1 mL water), 60% (0.3 mL kombucha + 0.2 mL water), 40% (0.2 mL kombucha + 0.3 mL water), and 20% (0.1 mL kombucha + 0.4 mL water). These were placed in 5 mL vials for each dilution and homogenized using a vortex mixer. The second stage involved making eight wells in each dish containing NA medium with a diameter of 6 mm, then swabbing the medium surface with *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 bacterial suspensions using a sterile cotton swab and leaving it for 5 min. Both test bacteria were chosen for their good antibacterial activities in the previous step. Amoxicillin was used as a positive control. All Petri dishes containing the test samples were incubated in an incubator for 24 h at 37 °C. The treatment was repeated thrice. After incubation for 24 h, the diameter of the inhibition zone formed on the medium in each well was measured using a caliper.

Determination of Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) assay was performed according to research conducted by Inanta (2023) using the spread plate method. Based on the results of the MIC assay, the MBC assay was conducted with the dilution that showed the best inhibition against the two bacteria, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591. Fifty microliters of each freshly cultured bacteria were inoculated into torch ginger kombucha dilution and homogenized. The tube test was incubated at 37 °C for 24 h.

From the dilution, the spread plate method was applied by taking 100 µL of each dilution and dripping it onto a Petri dish containing 20 mL of NA medium (for bacteria) and spreading it evenly using an L-rod (triangular rod). All petri dishes containing the test samples were incubated at 37 °C for 24 h. The concentration of the test material that can kill bacteria was determined

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by observing and counting the number of colonies that grew on each dish. The determination of the Minimum Bactericidal Concentration (MBC) is based on the smallest number of microbes, so it can be stated that this concentration can kill the test bacteria.

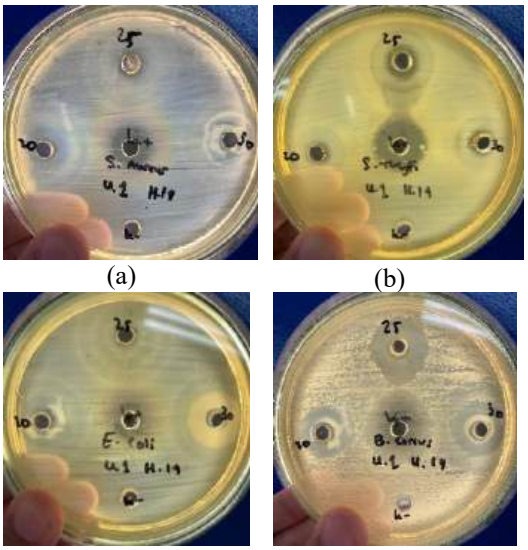
RESULTS AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATTC 14028 was assessed using the well diffusion method with sugar concentrations of 20, 25, and 30% and fermentation periods of 7, 14, and 21 d. Antimicrobial activity was measured based on the size of the inhibition zone formed around the well containing the test sample. Antimicrobial inhibition power can be classified into four categories based on the Davis and Stout standard: very strong (≥ 20 mm), strong (10–19 mm), moderate (5–10 mm), and weak (< 5 mm) (Khanifah, 2022). This antimicrobial activity can also be formed because the pH in the sample also greatly affects the formation of the inhibition zone.

The results of the antimicrobial activity test show that torch ginger extract kombucha with different sugar concentrations and fermentation times can inhibit the growth of *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATTC 14028, as indicated by the presence of clear zones (inhibition zones) around the wells (Figure 1.).

The antimicrobial activity of torch ginger kombucha extract originates from torch ginger (*Etlingera elatior*) flowers, which contain antimicrobial compounds such as flavonoids, saponins, polyphenols, alkaloids, steroids, tannins, phytosteroids, and amino acids. (Bogoriani et al., 2022). These phytochemical compounds have the potential to act as natural antimicrobials against pathogenic bacteria, such as those tested in this study. The results show that the formation of acid during kombucha fermentation plays an effective role in suppressing the growth of microorganisms, thereby extending the shelf life and improving product safety by preventing spoilage and inhibiting pathogenic microorganisms. (Kostić et al., 2025).



(a) (b) (c) (d)

Figure 1. Inhibition Zone diameter of torch ginger Extract Kombucha after 14 d of fermentation. *Staphylococcus aureus* ATCC 33591 (a), *Salmonella typhi* ATTC 14028 (b), *Escherichia coli* ATCC 11775 (c), *Bacillus cereus* ATCC 11778 (d).

Based on the data and results of the discussion above, it can be concluded that the kombucha extract from torch ginger at 20% sugar concentration with a fermentation period of 14 days has consistent antimicrobial activity (inhibitory power) from the first to the third repetition, compared to other concentrations and fermentation periods. A fermentation period of 14 days was optimal and had the potential for strong inhibitory activity against the growth of *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 (Table 1.).

The gram-positive bacteria, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591, were the most sensitive to antimicrobial testing. Gram-positive bacteria have resistance to acidic conditions through a mechanism involving a proton pump that can balance the pH in the cell and the antimicrobial substrate. The antimicrobial activity is mainly influenced by low pH conditions due to the formation of acetic acid and the presence of catechin compounds

produced by bacteria and yeast in the SCOBY culture during the fermentation process, thereby inhibiting the growth of various gram-positive and gram-negative microorganisms (Coelho et al., 2022).

Tabel 1. The diameter (mm) of inhibition zone of Kombucha from Torch ginger Extract on Pathogenic Bacteria

Sugar Concentration (%)	Fermentation Time (days)			Average
	7	14	21	
Bacillus cereus				
20	11.74±1.92	15.44±2.40	10.74±5.67	12.64±3.87
25	7.35±5.87*	17.69±3.33	14.12±8.60	13.06±7.11
30	9.40±5.25	18.03±5.81**	14.62±8.20	14.02±6.81
Average	9.50±4.48a	17.06±3.76b	13.16±6.83a	
K+	25.55±6.31	22.61±4.87	9.91±4.56	
K-	0.00±0.00	0.00±0.00	0.00±0.00	
Escherichia coli				
20	10.63±1.21c	14.03±2.60Ab	7.37±2.10a	10.67±1.97
25	8.42±0.55a	21.93±1.69Bb	6.23±2.76a	12.19±1.67
30	9.85±1.78b	22.85±0.54Bc**	4.80±1.49Ca*	12.50±1.27
Average	7.27±1.19b	13.18±1.19c	4.43±1.63a	
K+	7.46±2.43	7.12±3.58	3.79±1.48	
K-	0.00+0.00	0.00+0.00	0.00+0.00	
Staphylococcus aureus				
20	13.56±2.25**	10.94±7.47Aa	6.13±8.65*	10.21±6.12
25	11.82±6.07	12.89±10.14Aa	8.18±7.33	10.96±7.85
30	13.28±6.01	9.82±4.33Aa	8.96±7.82	10.69±6.05
Average	12.89±2.94	11.22±4.44	7.76±4.59	
K+	8.96±4.20	11.15±1.11	6.35±1.80	
K-	0.00±0.00	0.00±0.00	0.00±0.00	
Salmonella typhi				
20	13.33±4.10	9.11±5.64*	13.61±8.69	12.01±6,14
25	12.15±5.83	14.70±10.60	15.43±9.28	14.09±8,57
30	15.25±5.50	16.07±12.97	16.11±10.60**	15.81±9,69
Average	13.58±3.01	13.29±5.89	15.05±5.52	
K+	12.00±3.56	14.37±2.89	13.05±3.52	
K-	0.00±0.00	0.00±0.00	0.00±0.00	

Note: Data (mean± SD) were obtained from three replicates. Data were analyzed using analysis of variance (ANOVA). The data in the columns and rows with a gray background are those on the effects of sugar concentration and fermentation duration. The data in the areas with a white background represents the interaction between the treatments. Interaction data in the same row followed by different capital letters indicate an effect on different sugar concentrations, whereas interaction data in the same column followed by lowercase letters indicate an effect on fermentation time (DMRTα 5% test). The asterisks in the table indicate the highest (**) and lowest (*) inhibition zone results. Amoxycillin was used as a positive control.

Antimicrobial activity testing in this study used amoxicillin as a positive control for pathogenic bacteria. The positive control in this test was used to compare the inhibitory effects of the kombucha samples. Amoxicillin is a broad-spectrum antibiotic that can inhibit and kill both gram-positive and gram-negative bacteria. Resistance may occur because this antibiotic is widely used in the medical world for treatment. Antibiotic resistance can spread among bacteria through various channels, causing previously susceptible bacteria to become resistant to antibiotics. (Halawa et al., 2023).

Minimum Inhibitory Concentration (MIC)

The determination of the inhibition zone in the previous stage of the best treatment for further MIC testing was performed on *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 bacteria with a sugar concentration of 20% and a fermentation period of 14 d, as shown in Table 2 for the data obtained in this MIC test. This MIC method uses the well-diffusion

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method, but the samples to be tested must first be diluted. The dilutions used in this MIC test were original (100%), 80%, 60%, 40%, and 20%. The inhibition zones were shown in Figure 2.

Table 2. MIC Determination of *Bacillus cereus* and *Staphylococcus aureus*

Kombucha Dilution Concentration (%)	Inhibition zone (mm)		MBC Assay (mm)	
	B. cereus ATCC 11778	*S. aureus* ATCC 33591	*B. cereus* ATCC 11778	*S. aureus* ATCC 33591
100	6.73	0.90	214.50	287.50
	1.13	0.50	89.00	75.50
	11.80	0.83	80.00	3.00
80	4.63	0.70	50.50	83.00
	0.80	0.40	54.50	398.00
	9.37	0.43	90.00	4.50
60	0.50	0.67	421.50	58.50
	0.50	0.00	286.00	0.00
	6.13	0.00	71.00	0.00
40	0.70	0.00	68.50	0.00
	0.60	0.00	171.50	0.00
	0.80	0.00	51.00	0.00
20	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
Average	2.91	0.30	109.87	60.67
SD	3.85	0.35	117.75	120.09

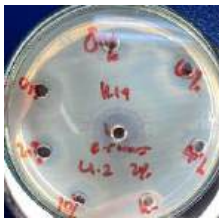
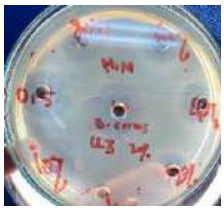
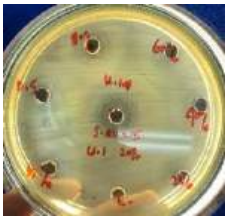
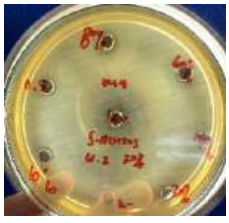
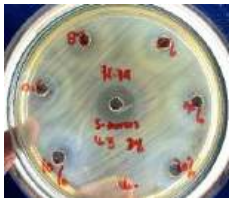
Bacterial type	Repetition		
	1	**2**	**3**
Bacillus cereus ATCC 11778			
Staphylococcus aureus ATCC 33591			

Figure 2. Width clear zones of the MIC assay on *B*. cereus and *S. aureus* ATCC 33591 at 14 d fermentation period.

Based on the data and results of the discussion in the table and figure above, *Bacillus cereus* ATCC 11778 showed no bacterial growth at 40% and 20% concentrations. *Staphylococcus aureus* ATCC 33591 at 80, 40, and 20% concentrations

showed no bacterial growth. The lowest concentration at which no bacterial growth was observed was defined as the Minimum Inhibitory Concentration (MIC). Therefore, the Minimum Inhibitory Concentration (MIC) value for the *Bacillus cereus* ATCC 11778 bacteria is at a concentration of 40%. The Minimum Inhibitory Concentration (MIC) value for *Staphylococcus aureus* ATCC 33591 bacteria is at a concentration of 80%. The Minimum Inhibitory Concentration (MIC) value is indicated by the absence of an inhibition zone around the well at the concentration that has been made. The inhibition of test microbe growth can be caused by active compounds such as tannins, essential oils, flavonoids, and anthocyanins, which are found in torch ginger extract. This can result in a reduction in the availability of nutrients for bacteria, so that bacteria cannot synthesize DNA, peptidoglycan, and other processes necessary for their growth. This can result in a decrease in the ability of bacteria to adhere to surfaces and inhibit the synthesis necessary for cell wall formation (Inanta, 2023).

Minimum Bactericidal Concentration (MBC)

The MBC was determined based on the best inhibition observed in the MIC assay. The MBC test aimed to determine the concentration that could kill the microbes being tested. The MBC method uses the spread plate method, whereby the sample to be tested is first diluted. The dilution used in this MBC test was *Staphylococcus aureus* ATCC 33591 bacteria. The solution samples selected for the MBC test were 100%, 80%, and 60% (replication 1) concentrations. For replication 2 and 3, the solution samples selected for the MBC test were 100% and 80%, respectively. For *B. cereus* ATCC 11778, the solution samples selected for the MBC test were 100, 80, 60, and 40% for replicates 1 to 3, respectively (Figure 3.).

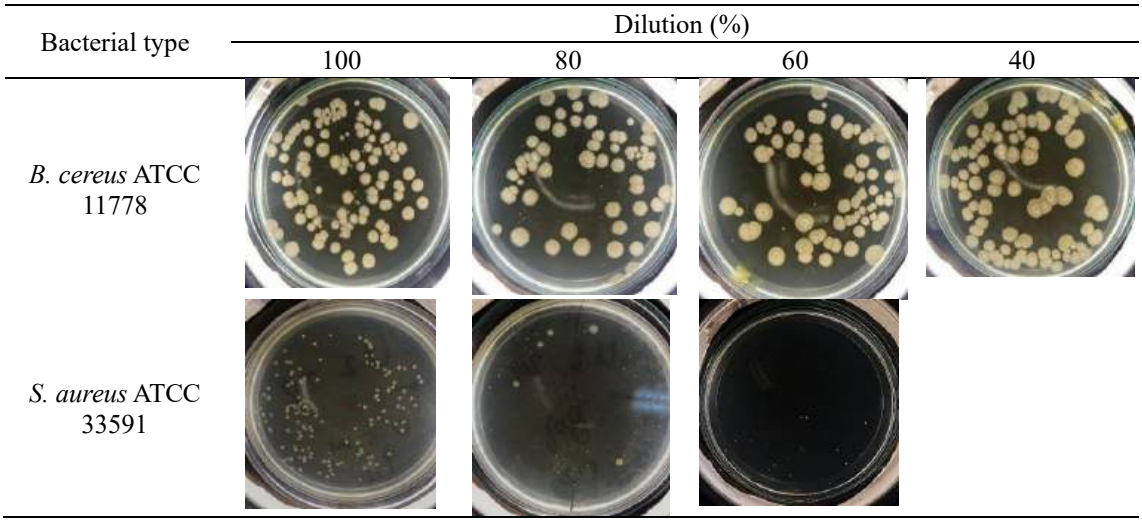


Figure 3. Colony growth in the MBC assay of *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591

The data obtained showed that torch ginger leaf and flower extracts have significant antibacterial activity against gram-positive bacteria. This is in line with the results of the MIC and MBC tests on kombucha samples made from torch ginger extract, which confirmed the ability of this fermented drink to inhibit and inactivate the growth of *Bacillus cereus* and *Staphylococcus aureus*. These findings indicate that torch ginger extract kombucha has the potential to be developed as a natural antibacterial agent, with a mechanism of action that can be quantitatively measured using MIC and MBC values.

CONCLUSION

The results of the antimicrobial activity test to determine the inhibition zone showed that the effect of different sugar

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concentrations and fermentation times of torch ginger (*Etlingera elatior*) kombucha extract had a significant effect on the antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract on Escherichia coli ATCC 11775 bacteria, and Bacillus cereus ATCC 11778, while sugar concentration and fermentation duration did not significantly affect the antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract on Staphylococcus aureus ATCC 33591 and Salmonella typhi ATCC 14028 bacteria.

The minimum inhibitory concentration (MIC) test was conducted on Bacillus cereus ATCC 11778 and Staphylococcus aureus ATCC 33591 bacteria with a sugar concentration of 20% and a fermentation period of 14 days. The Minimum Lethal Concentration (MLC) test on Bacillus cereus ATCC 11778 bacteria yielded results at a dilution concentration of 10%. Staphylococcus aureus ATCC 33591 yielded results at a dilution of 20%.

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