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Total Phenolic Content and Hedonic Quality of Germinated Jack Bean Tempeh at Different Fermentation Times and Packaging Types

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ABSTRACT
This study evaluated the interaction effects of different fermentation durations and packaging types on producing the best-germinated jack bean tempeh based on total phenolic content (TPC) and hedonic quality. This study uses a factorial experimental design with a randomized group of 3 × 3 treatments. The factors included fermentation durations (48, 60, and 72 hours) and packaging types (plastic, banana leaves, and teak leaves). There were 9 treatments with 3 replications each. Statistical analysis for TPC was performed using ANOVA, while hedonic quality was analyzed using the Friedman test. If significant effects ($p<0.05$) were detected, data analysis was continued with DMRT. The best treatment was determined using De Garmo's effectiveness index test. The interaction between fermentation duration and packaging type significantly affected TPC ($p=0.045$), color quality ($p=0.000$), aroma ($p=0.004$), and appearance ($p=0.000$), but had no significant effect on taste quality ($p=0.100$). The best treatment was tempeh which had a fermentation duration of 60 hours and used plastic packaging. It contained 0,97 mg GAE/g of phenolic compounds (low) with antioxidant activity IC_{50} value of 6.951,38 ppm (weak). It had hedonic scores of 3.50 for taste (characteristic of tempeh), 3.20 for aroma (not musty), 3.90 for color (white), and 3.20 for appearance (compact). Based on its TPC and antioxidant activity, it is not sufficiently strong to inhibit oxidative stress. These results can be used as a basis for considering the use of germinated jack beans as an alternative antidiabetic functional food product.

KEYWORDS
Fermentation duration; Germinated jack bean tempeh; Hedonic quality; Packaging type; Total phenolic content

1. INTRODUCTION

Tempeh is a functional food with an affordable price that is widely consumed as vegetable protein. In Indonesia, around 81,000 tempeh manufacturing businesses produce 2.4 million tons of tempeh per year [1], while the amount of tempeh consumption reaches 7.47 kg per capita per year [2]. Tempeh is usually made from soybeans fermented using *Rhizopus spp*. mold [3]. The low supply and productivity of soybeans in Indonesia encourage soybean imports. Therefore, it is necessary to find substitute materials with similar or even higher functional potential to reduce Indonesia's dependence on imported soybeans. Tempeh is known as a superfood, because the manufacturing process involves various microorganisms (lactic acid bacteria, mold, yeast, and others) through a fermentation process (solid-state fermentation) which forms the nutrients and the bioactive compounds of health importance [4], such as antioxidants [5].

Foods rich in antioxidants can overcome oxidative stress, suppress inflammatory markers, and control blood glucose levels. One type of antioxidant compound that can play a role is phenolic compounds [6]. Soybean tempeh is high in phenolic compounds [7]. One of the food sources of antioxidants, especially

phenolics, which has the potential to be used as a substitute for soybeans in tempeh is jack beans (*Canavalia ensiformis* (L.) D.C.). Jack beans contain 32.32% protein, 61.15% carbohydrates, and 2.89% fat [8]. Jack beans is one type of alternative local material to increase protein with a high protein content [9]. The use of jack beans as substitute material to increase protein levels has been widely carried out [8], [10]. Jack bean tempeh contains 0.78% isoflavone antioxidant compounds. Isoflavones are flavonoid derivative compounds which are one of the polyphenol compound groups [11]. The production level of jack beans in Indonesia is relatively high, but their use as ingredients in food products is still rare.

Increasing isoflavones in jack beans can be done by germination [12]. Germination treatment can increase antioxidant activity because there is a process of changing isoflavone glycosides into aglycone compounds in the form of daidzein, genistein, and glycitein [13]. In addition to improving the quality of isoflavones, germination can also reduce the levels of anti-nutritional compounds, such as hydrocyanic acid (HCN). The HCN levels in jack bean sprout flour decreased significantly after germination for 60 and 72 hours, namely 9.62 ppm and 8.95 ppm (<10 ppm), so it is safe for consumption [14].

In addition to germination, the fermentation process can also affect antioxidant activity [15]. Previous study highlights the highest antioxidant activity of black soybean tempeh occurred when the fermentation time was 42 hours, at 67.40% [13]. During fermentation, there was further conversion of glycitein and daidzein into Factor-II compounds (6,7,4' trihydroxy isoflavones) which have higher antioxidant activity [16]. Germination and fermentation treatments can be given to jack beans as raw material for making tempeh.

The type of tempeh packaging is one of the things that needs to be considered. The quality or hedonic quality of tempeh can be influenced by the type of tempeh packaging. The type of packaging affects the hedonic quality of tempeh, including the taste, aroma, and texture of soybean tempeh, but does not affect the color quality [17], [18]. The type of packaging is also one of the factors that affect the growth of mold during the fermentation process. Mold can grow better in leaf packaging, because it is light-proof and has good aeration to maintain humidity during the fermentation process [19]. Commonly used natural leaf packaging includes banana leaves, teak leaves, and waru leaves. In addition, the water content of tempeh is higher in banana leaf packaging than plastic, because banana leaves can maintain the water content in tempeh [20]. Leaf packaging produces a distinctive aroma, because there is polyphenol content in the leaves, but people prefer to use plastic packaging because it is more practical. However, the use of plastic packaging needs to be perforated because it has low air, vapor, and heat permeability [21].

Currently, the consumption of non-soybean tempeh is still less popular, so a study of functional properties, such as total phenolic tests on tempeh needs to be conducted. This is expected to be one of the efforts to increase consumer interest in non-soybean tempeh, one of which is germinated jack bean tempeh. Germination and fermentation treatments on jack beans are expected to produce food products in the form of tempeh that are rich in phenolic compounds. Different fermentation times and types of packaging have the potential to produce germinated jack bean tempeh with different TPC and hedonic quality. There has been no research that proves that different fermentation times and types of packaging can affect these variables. Therefore, this study aims to determine the effect of different fermentation times and types of packaging on the TPC and hedonic quality of germinated jack bean tempeh as a food that has the potential to be antidiabetic.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in making germinated jack bean tempeh are jack beans (*Canavalia ensiformis* (L.) D.C.) (from Wonogiri farmers, Indonesia), yeast (Raprima, Indonesia), and various types of packaging (plastic, banana leaves, and teak leaves). The chemicals used for analysis include 95% methanol (Merck, USA), Folin Ciocalteu reagent (Merck, USA) and sodium carbonate (Na_2CO_3) (Merck, USA).

2.2. Jack Bean Sprouts Preparation

Jack bean sprout production follows the method of Agustia et al., 2023 [14]. Jack bean seeds are washed thrice and added with $NaHCO_3$ solution (1% w/w) (temperature 50 °C, water:jack bean seeds = 5:1

v/w) for 24 hours. The soaking water is changed every six hours. After 24 hours of soaking, the jack bean seeds are rinsed three times, then placed and arranged neatly on a tray lined with a wet cloth. Germination is carried out in a dark and room temperature, and covered with a wet cloth, but the wet cloth should not drip water. The covering cloth is changed every 6 hours. The germination process is carried out for 60 hours.

2.3. Germinated Jack Bean Tempeh Preparation

The preparation of germinated jack bean tempeh follows the method of Puspitojati et al., 2019 [23] with modifications. Germinated jack beans were washed twice, and the skin was peeled, then boiled for 30 minutes (water:sprouts = 4:1 v/w) and drained. After draining, the germinated jack beans were sliced into four to six pieces and soaked again for 24 hours (water:sprouts = 4:1 v/b). After soaking, the second boiling was carried out with the same time and amount of water as the first boiling. After draining and cooling, the pieces of germinated jack beans were given 0.2% (w/w) yeast for the fermentation process. After fermentation, it was packaged in three packaging types: plastic, banana leaves, and teak leaves. The final stage in making germinated jack bean tempeh was fermentation for 0–72 hours at room temperature, and the changes were observed at three different times, 48, 60, and 72 hours.

2.4. Total Phenolic Content (TPC) Analysis

The analysis of TPC used a powdered sample of germinated jack bean tempeh. The germinated jack bean tempeh was thinly sliced and dried in a drying cabinet at a temperature of 55 °C for 6 hours. The dried germinated jack bean tempeh was ground in a food grinder for two minutes per sample unit and sifted through a 60-mesh sieve. The germinated jack bean tempeh powder was used for the TPC analysis.

Preparing the gallic acid standard curve begins with making a stock solution of 1000 ppm gallic acid. From the stock solution, a series of concentrations of 0, 25, 50, 100, 150, and 200 ppm is made. A total of 0.1 mL of each concentration of a standard solution is put into a test tube. Each test tube is added with 5 mL of distilled water reagent and 0.2 mL of Folin-Ciocalteu reagent that has been diluted with distilled water in a ratio of 1:1. All mixtures in each test tube are shaken until mixed well, then left for 5 minutes. Then, 1 mL of 5% Na_2CO_3 solution is added and vortexed, then left for 1 hour in the dark. One hour of leaving after, the absorbance of all concentrations of the standard solution is read by a UV-Vis spectrophotometer at a wavelength of 747 nm. The results of the absorbance measurements are used to create a calibration graph so that the relationship between gallic acid concentration (mg/mL) and absorbance can be determined.

Analysis of TPC in samples follows the method by Orak et al., (2007) [24]. A total of 1 g of tempeh powder is diluted with 10 mL of distilled water, then filtered. Following filtration, 0.1 mL of the sample filtrate is put into a test tube, then 5 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent that has been dissolved in distilled water in the ratio 1:1, shaking until well combined. After the solution in the test tube is evenly mixed, it is then left for 5 minutes. A total of 1 mL of 5% Na_2CO_3 is added and stirred, then left for 1 hour in the dark. Measurement of the absorbance of the solution is measured in a UV-Vis spectrophotometer at a predetermined maximum wavelength of 747 nm. Absorbance measurements are carried out twice to obtain accurate results. After measuring the absorbance, the TPC is calculated using the equation (1), where C is the phenolic concentration (x value), V is the volume of extract used (mL), fp is a dilution factor, and g is weight of sample used (g).

$$Total\ phenolic\ content\ (TPC) = \frac{C \times V \times fp}{g} \quad (1)$$

2.5. Hedonic Quality Testing

The hedonic quality testing of germinated jack bean tempeh products was carried out using a hedonic quality test form with four test attributes, such as color, aroma, appearance (mycelia compactness), and taste. Assessment of color, aroma, and appearance was carried out by organoleptic test, while the taste

assessment was carried out on germinated jack bean tempeh that had been steamed for 10 minutes. These hedonic test attributes are assessed on a Likert scale of 1–5 with the provisions in Table 1.

Table 1. Hedonic quality test scale.

Color	Aroma	Appearance	Taste	Score
Not white	Very beany	Not compact	Not typical tempeh	1
Less white	Beany	Less compact	Less typical tempeh	2
Quite white	Quite beany	Quite compact	Quite typical tempeh	3
White	Less beany	Compact	Typical tempeh	4
Very white	Not beany	Very compact	Very typical tempeh	5

Hedonic quality testing was conducted by 50 untrained panellists [25]. The panellists were selected based on several criteria. Some of these criteria include being willing to participate in the hedonic quality test; being in good health, having no history of allergies, and color blindness; not smoking or consuming alcohol; and not consuming food or drinks within 1 hour before the hedonic test. This hedonic quality test has received ethical approval from the Health Research Ethics Commission of the Faculty of Health Sciences, Jenderal Soedirman University, Number 1597/EC/KEPK/X/2024.

2.6. Best Treatment Determination

The best treatment is determined according to the effectiveness index method of DeGarmo et al., (1977) [26]. The variable weight value (BV) is determined for each parameter with a relative number between 0 and 1. The weight value is given based on the importance of each parameter in achieving the results due to the treatment. The higher the importance level, the higher the variable weight value. The normal parameter value weight (BN) is calculated by dividing BV by the total treatment variable weight (BVT), while the effectiveness value (NE) is calculated by dividing the difference between the treatment value (NP) and the worst value (NBr) by the difference between the best value (NBk) and worst values (NBr). The result value (NH) is calculated for all parameters by multiplying NE with BN. The best treatment is identified based on the treatment with the highest result value (NH). The effectiveness value can be calculated by equation (2) and the best treatment determination can be calculated by equation (3).

$$Effectiveness\ value\ (NE) = \frac{treatment\ value\ (NP) - worst\ value\ (NBr)}{best\ value\ (NBk) - worst\ value(NBr)} \quad (2)$$

$$The\ result\ value\ (NH) = effectiveness\ value\ (NE) \times normal\ weight\ (BN) \quad (3)$$

2.7. IC_{50} Antioxidant Activity Testing

The IC_{50} antioxidant activity test for the best treatment was carried out using the method by Molyneux, (2004) [27]. Samples were made in a series of concentrations of 100–500 ppm. A total of 1 mL of each stock solution was put into a different test tube, and 1 mL of 200 µM DPPH solution was added, then incubated for 30 minutes in a dark room. The solution was diluted using ethanol to 5 mL. Each sample and blank solution was calibrated (its absorbance was measured) at a wavelength of 517 nm. The determination of the percentage (%) inhibition used the equation (4), where A is the absorbance of the blank, B is the absorbance of the sample.

$$Inhibition\ (\%) = \frac{A - B}{A} \times 100\% \quad (4)$$

A straight-line equation y = ax + b were made with the provisions of the x axis as the concentration (ppm) and the y axis as the %inhibition. Then the value of x (IC_{50}) is calculated by changing y with a value

of 50, so that the formula for determining the IC_{50} value is obtained from equation (5), where a is the intercept, and b is the slope.

$$IC_{50} = \frac{50 - b}{a} \times 100\% \quad (5)$$

2.8. Experimental Design

This study used a factorial experimental research method with a 3 × 3 factorial design and used the Randomized Block Design (RBD). The treatments given consisted of two treatment factors. Each treatment factor has three levels, namely fermentation time (L1 = 48 hours; L2 = 60 hours; L3 = 72 hours) and type of packaging (J1 = plastic; J2 = banana leaves; J3 = teak leaves) [19], [22]. Testing for each treatment was carried out with three replications so that a total sample of 27 experimental units was obtained. The study began with jack bean sprouts preparation, germinated jack bean tempeh preparation, germinated jack bean tempeh flour preparation, and continued with total phenolic and hedonic analysis.

2.9. Data Analysis

Data analysis in this study was conducted using SPSS IBM 26. The data analysis method used for TPC was the ANOVA test with α = 5%, while the hedonic quality was analyzed using the Friedman test. If there was a significant effect, the data analysis was continued with Duncan's Multiple Range Test (DMRT). Furthermore, the DeGarmo effectiveness index test method was conducted to determine the best treatment for germinated jack bean tempeh.

3. RESULTS AND DISCUSSION

3.2. Total Phenolic Content (TPC)

Phenolic compounds are the largest group of compounds in plants that function as natural antioxidants [28]. The TPC of germinated jack bean tempeh at different fermentation times and packaging types can be seen in Figure 1. TPC analysis was conducted on germinated jack bean tempeh fermented for 48, 60, and 72 hours. The fermentation period of 48–72 hours was chosen because the results of preliminary studies showed that optimal growth of tempeh mold occurred during that period time. This is supported by research on jack bean tempeh which states that mycelium can cover the entire surface of tempeh and can maintain the structure of tempeh at the 48–72 hours fermentation time [23]. The study also stated that after a fermentation period of more than 72 hours, the quality of mold growth began to decline. Based on Figure 1, it is known that the TPC of tempeh packaged using plastic, banana leaves, and teak leaves increased significantly at a fermentation period of 48–72 hours. However, at a fermentation period of 48–60 hours, tempeh packaged using different types of packaging did not show a significant difference in TPC, a significant difference was only seen at a fermentation period of 72 hours.

The increase in TPC at a fermentation time of 48–72 hours is in line with the results of similar studies showing an increase in TPC at a fermentation time of 0–120 hours with TPC ranging from 2.49–10.70 mg GAE/g [29]. According to the study, during fermentation, glycosidic bonds are broken through a hydrolysis process that produces simpler phenolic monomers. Fermentation allows the degradation of complex compounds into simpler compounds, including the release of phenolic compounds bound to complex compounds. This can be caused by the enzymatic hydrolytic activity of *Rhizopus* sp. during fermentation [30]. *Rhizopus* sp can produce the enzyme β-glucosidase, which acts as a catalyst in the enzymatic hydrolytic process. This enzyme can hydrolyze phenolic glycosides and release them as free phenolic acids. [31]. In addition, fermentation has been shown to result in the formation of new bioactive compounds known as phenolic compounds, whereby the content of phenolic compounds is increased [32]. When these new phenolic compounds are formed, the TPC will also increase.

The increase in TPC can also occur due to the germination carried out on jack beans before fermentation. Germination of jack beans can activate endogenous enzymes such as phenylalanine ammonia lyase (PAL), which plays a role in the synthesis of phenolic compounds because it is directly bound to phenylalanine [33]. PAL is a catalyst in the elimination reaction of ammonia molecules from cinnamic acid

[34]. This reaction is a mechanism for the formation of secondary metabolite compounds from the phenolic group formed from phenylalanine.

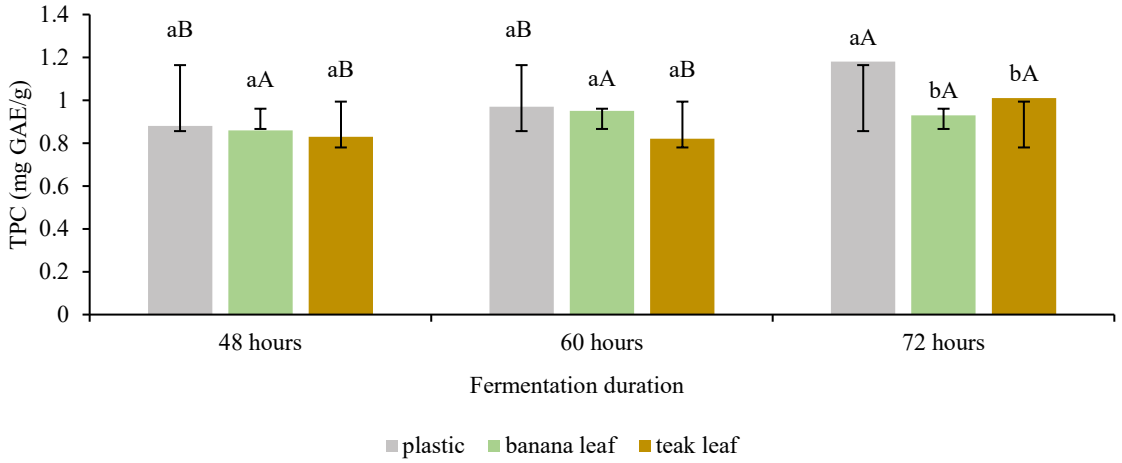

Figure 1. TPC of germinated jack bean tempeh at different fermentation times and packaging types.
Note: The difference in lowercase letters (a–b) in tempeh with the same fermentation time, but different packaging types, shows a significant difference (Duncan test, $p<0.05$). The difference in uppercase letters (A–B) in tempeh with the same type of packaging, but different fermentation times, shows a significant difference (Duncan test, $p<0.05$).

The TPC of tempeh packaged using plastic, banana leaves, and teak leaves at the same fermentation time did not show any significant differences, presumably because the three types of packaging have the same good aeration to maintain humidity and support mold growth. The type of packaging is one of the factors that influence mold growth because mold growth requires oxygen (aerobic) [35]. Previous studies have shown that mold can grow better on leaf packaging than plastic because leaf packaging is light-tight and has pores that support good aeration [19]. However, if the use of plastic packaging is given holes of sufficient size and number, aeration during fermentation can also run well [35]. Other studies have shown that there is no significant difference in the number of lactic acid bacteria (LAB) contained in tempeh, both those packaged using plastic (given holes) and several types of leaf packaging [36].

The highest TPC (1.18 mg GAE/g) was found in tempeh with a fermentation period of 72 hours and packaged using plastic. This is due to the interaction between the long fermentation time and the use of packaging materials that can support mold growth. The TPC in germinated jack bean tempeh is increased by fermentation time, because the development of the quantity of molds also rises [29]. In addition, the use of plastic packaging with holes of sufficient size and number also supports mold growth [35]. The lowest TPC (0.82 mg GAE/g) was found in tempeh with a fermentation period of 60 hours and packaged using teak leaves. These results are not in line with previous studies that TPC increases with increasing fermentation time [29]. This can occur due to the interaction between the fermentation period and the type of packaging. Although fermentation has been going on for 60 hours, the tempeh is packaged using teak leaves. Teak leaves have many pores for aeration of tempeh mold, but on the underside of the leaf surface there are hair glands and trichomes (fine hairs) which cause uneven mycelium growth [36].

3.3. Hedonic Quality

The effect of fermentation time and different types of packaging on the hedonic quality of germinated jack bean tempeh can be seen in Table 2. The results of Friedman's analysis showed that the fermentation time and different types of packaging significantly affected the quality of color, aroma, and appearance, but did not significantly affect the quality of taste. Color is a hedonic quality test parameter that is the first impression for panellists when seeing food products. The color of tempeh based on SNI 3144:2015 is white evenly on the entire surface of the tempeh [37]. The white color of tempeh arises due to the growth of mold mycelium that covers the surface of the tempeh [19]. Based on the panellists' assessment, germinated jack

bean tempeh which fermented for 48 hours has a very white color. This is thought to be because the fermentation time has not been too long, so the color of the tempeh looks very white. This is in line with previous research which stated that the color of tempeh is whiter in tempeh that has not been fermented for long, because the condition of the mold is still active during the growth period [19]. Longer fermentation can cause the color of tempeh to change to black due to uncontrolled microorganism activity [38].

Table 2. Hedonic quality of germinated jack bean tempeh.

Treatment	Color	Aroma	Appearance	Taste
L1J1	$4.10^{a} \pm 0.87$	$3.10^{ab} \pm 1.18$	$3.90^{a} \pm 0.91$	3.12 ± 0.87
L1J2	$3.72^{ab} \pm 0.90$	$3.26^{a} \pm 1.24$	$3.80^{ab} \pm 0.94$	3.12 ± 1.00
L1J3	$2.90^{de} \pm 0.93$	$3.28^{a} \pm 1.20$	$3.56^{abcd} \pm 1.01$	2.96 ± 1.09
L2J1	$3.50^{bc} \pm 0.89$	$3.20^{a} \pm 1.37$	$3.90^{a} \pm 0.85$	3.20 ± 0.97
L2J2	$2.40^{f} \pm 1.03$	$2.50^{b} \pm 1.21$	$3.16^{d} \pm 1.04$	2.60 ± 1.13
L2J3	$2.42^{f} \pm 0.81$	$3.36^{a} \pm 0.99$	$3.40^{bcd} \pm 0.88$	2.84 ± 1.14
L3J1	$3.50^{bc} \pm 0.86$	$3.00^{ab} \pm 1.31$	$3.60^{abc} \pm 1.07$	2.96 ± 1.05
L3J2	$3.20^{cd} \pm 0.96$	$3.10^{ab} \pm 1.30$	$3.62^{abc} \pm 0.97$	3.20 ± 1.06
L3J3	$2.70^{ef} \pm 1.07$	$3.48^{a} \pm 1.17$	$3.20^{cd} \pm 1.02$	2.90 ± 1.23

Note: L = Fermentation time (1, 48 hours; 2, 60 hours; 3, 72 hours); J = Packaging types (1, plastic; 2, banana leaf; 3, teak leaf). Different letter notations indicate significant differences in the same column by the DMRT test ($p<0.05$).

In addition to the fermentation time, the color of tempeh is also influenced by the type of packaging used. Based on the results of the study, the highest value given by the panellists for color quality was found in tempeh packaged using plastic. This is not in line with previous studies which stated that gude tempeh packaged using leaves has a whiter color, because leaf packaging is light-proof [19]. However, a person's acceptance of the color of tempeh is likely influenced by various factors. Previous studies have stated that the acceptance of food color is influenced by several factors, including natural conditions, geography, and social aspects of the recipient community [39].

Aroma is a hedonic quality test parameter that uses the sense of smell to determine how panellists or consumers accept food products [40]. The aroma of tempeh based on SNI 3144:2015 is the typical aroma of tempeh without the smell of ammonia [37]. The typical aroma of tempeh appears due to the fermentation process, but in germinated jack bean tempeh, a rancid aroma can also be found which appears from free amino acids and fat decomposition during fermentation [19]. In addition, the rancid aroma is also a typical aroma found in jack beans, but the rancid aroma can be reduced if soaked in a sodium bicarbonate ($NaHCO_3$) solution [41]. Based on the panellists' assessment, germinated jack bean tempeh with a fermentation period of 72 hours has a non-rancid aroma that is preferred. This is thought to be because the longer fermentation causes the typical rancid aroma of jack beans to decrease. Fermentation can reduce the concentration of compounds that cause the unpleasant aroma in jack beans [42]. In addition, the aroma of tempeh is also influenced by the type of packaging used. Based on the results of the study, the highest value given by panellists for aroma quality was found in tempeh packaged using teak leaves. Tempeh packaged using leaves has a distinctive aroma because leaves contain polyphenols [19]. However, this is not in line with the results of other studies which state that panellists prefer tempeh packaged using plastic because it has a stronger distinctive tempeh aroma [18], [36].

The appearance (mycelia compactness) of tempeh can be seen from the mycelium that covers the jack beans [40]. The appearance or texture of tempeh based on SNI 3144:2015 is compact mycelium, when sliced it remains intact (does not fall off easily) [37]. The compact appearance of tempeh can be seen from the growth of mold mycelia that connects the jack bean seeds [43]. The panelists thought that after 48 hours of fermentation, the mycelium of germinated jack bean tempeh looked the most dense.This is thought to be because when the hedonic quality test was carried out, the presentation of the tempeh was cut into small pieces so that the panellists had difficulty seeing the compactness of the mycelia in the tempeh. The results of this study are also not in line with previous studies on white sword bean tempeh. The study stated that

tempeh with a longer fermentation time had a more compact appearance, namely at a fermentation time of 60–72 hours [23]. The longer the fermentation takes place, the more mycelia are formed [13].

In addition to the fermentation time, the appearance of tempeh is also influenced by the type of packaging used. Based on the results of the study, the highest value given by the panellists for appearance quality was found in tempeh packaged using plastic. This is not in line with previous studies which stated that mold can grow better on leaf packaging than plastic, because leaves have many pores for aeration during fermentation [19]. Although plastic packaging is airtight, providing holes of sufficient size and number can make aeration during fermentation also run well [35]. Therefore, the appearance of germinated jack bean tempeh packaged using plastic can be more compact than banana leaf and teak packaging. Taste is a stimulus received by the sense of taste after tempeh is eaten [43]. Different fermentation times and types of packaging do not significantly affect the taste of germinated jack bean tempeh. This can happen because before being served to the panellists, the tempeh is steamed for 10 minutes, so the taste of one tempeh and another may be difficult to distinguish. The taste of tempeh is not only determined by the raw materials but also by the production process, including how the tempeh is processed before being served to the panellists [36]. In addition, a person's acceptance of the quality of taste is likely influenced by various factors. Acceptance of the taste of tempeh is influenced by many factors, such as age, gender, habits, and lifestyle [36].

3.4. Best Treatment Determination

The optimal treatment for germinated jack bean tempehat different fermentation times and packaging types was determined using the effectiveness index method [26]. This determination was based on factors such as phenolic content and sensory qualities, which include color, aroma, appearance (mycelial compactness), and taste. Each parameter is given a weight with a relative number between 0 and 1. The Value Weight (BV) is given based on the importance of each parameter in producing the final product of germinated jack bean tempeh which has the potential as an antidiabetic. The value weights assigned to each parameter in this study are presented in Table 3.

Table 3. Value weight (BV).

Parameter	BV
Total phenolic content	1
Taste	0.9
Aroma	0.8
Color	0.7
Appearance	0.6
Total value weight (BVT)	4

Total phenolic content is given the highest value, which is 1, because germinated jack bean tempeh is superior in its antioxidant content, especially phenolics which can act as antidiabetics. Phenolics can protect β-pancreatic cell damage due to free radicals, inhibit Glucose Transporter type-2 (GLUT-2), and reduce oxidative stress [44], [45]. Taste quality is given a value of 0.9 because taste is an important aspect considered by consumers in choosing food products. Even though a food product has good nutritional value, the product cannot be accepted by consumers if the taste is not liked [46], [47]. Aroma is given a value of 0.8 because aroma can make consumers more comfortable in consuming a food product. Aroma has a significant influence in shaping consumer perceptions of a food product [48]. Color is given a weighting value of 0.7 because consumers tend to choose food products that have visual appeal, one aspect of which is attractive color. In addition, color also affects the perception of taste and aroma, because consumers often judge the taste of a food product based on the color they see [49]. Appearance (mycelia compactness) is given the lowest weighting value, namely 0.6, because consumers tend to pay more attention to other factors when choosing food products in the form of tempeh, such as taste and aroma rather than specific details related to the compactness of tempeh mycelia [43]. After giving the weighting value, the next step is to

calculate the Parameter Value Weight (BN). The results of this calculation obtained the weighted value for each parameter in Table 4.

Table 4. Parameters value weight (BN).

Parameter	BN
Total phenolic content	0.25
Taste	0.23
Aroma	0.20
Color	0.18
Appearance	0.15
Total BN	1

After calculating the BN, the next step is calculating the Effectiveness Value (NE). The treatment value, the best value, and the worst value of each test parameter on white sword bean sprout tempeh can be seen in Table 5.

Table 5. Average value, highest value, and lowest value.

Treatment	Phenolic content	Taste	Aroma	Color	Appearance
L1J1	0.88	4.10	3.10	3.90	3.12
L1J2	0.86	3.72	3.26	3.80	3.12
L1J3	0.83	2.90	3.28	3.56	2.96
L2J1	0.97	3.50	3.20	3.90	3.20
L2J2	0.95	2.40	2.50	3.16	2.60
L2J3	0.82	2.42	3.36	3.40	2.84
L3J1	1.18	3.50	3.00	3.60	2.96
L3J2	0.93	3.20	3.10	3.62	3.20
L3J3	1.01	2.70	3.48	3.20	2.90
The best value (NBk)	1.18	3.2	3.48	4.1	3.9
The worst value (NBr)	0.82	2.6	2.5	2.4	3.16
Difference	0.36	0.6	0.98	1.7	0.74

The results of the calculation of the effectiveness value (NE) of each test parameter on germinated jack bean tempeh can be seen in Table 6. After calculating NE, the next step is to calculate the result value (NH). The NH calculation can be seen in Table 7. The highest NH calculation results determine the best treatment of germinated jack bean tempeh. Based on the results above, the best treatment for germinated jack bean tempeh at different fermentation times and packaging types is L2J1 (fermentation time 60 hours and plastic packaging type). L2J1 has the highest NH of 0.74. L2J1 has 0.97 mg GAE/g phenolic content, and a taste quality score of 3.50; aroma 3.20; color 3.90; and appearance 3.20.

Table 6. Effectiveness value (NE).

Treatment	Phenolic content	Taste	Aroma	Color	Appearance
L1J1	0.17	0.87	0.61	1.00	1.00
L1J2	0.11	0.87	0.78	0.78	0.86
L1J3	0.03	0.60	0.80	0.29	0.54
L2J1	0.42	1.00	0.71	0.65	1.00
L2J2	0.36	0.00	0.00	0.00	0.00
L2J3	0.00	0.40	0.88	0.01	0.32
L3J1	1.00	0.60	0.51	0.65	0.59
L3J2	0.31	1.00	0.61	0.47	0.62
L3J3	0.53	0.50	1.00	0.18	0.05

Phenolic compounds are the largest group of plant components with natural antioxidant capacity [28]. Food products containing phenolics can potentially be antidiabetic because one of the triggers for Type 2 DM is oxidative stress [50], [51]. Germinated jack bean tempeh contains phenolics that are thought to have the potential to be antidiabetic, but L2J1 only contains phenolics of 0.97 mg GAE/g (<10 mg GAE/g) which is included in the low category [52].

Table 7. The result value (NH).

Treatment	Phenolic content	Taste	Aroma	Color	Appearance	Total
L1J1	0.74	0.20	0.12	0.18	0.15	0.68
L1J2	0.03	0.20	0.16	0.14	0.86	0.64
L1J3	0.01	0.14	0.16	0.05	0.54	0.43
L2J1	0.10	0.23	0.14	0.11	1.00	0.74
L2J2	0.09	0.00	0.00	0.00	0.00	0.09
L2J3	0.00	0.09	0.18	0.00	0.32	0.32
L3J1	0.25	0.14	0.10	0.11	0.59	0.69
L3J2	0.08	0.23	0.12	0.08	0.62	0.60
L3J3	0.13	0.11	0.20	0.03	0.05	0.48

Based on the results of the hedonic quality test, germinated jack bean tempeh with the best treatment (L2J1) has a good value according to the panellist's evaluation. The average value given by the panellists for the hedonic quality of L2J1 tempeh is color 3.90 (white), aroma 3.20 (not beany), appearance 3.20 (compact), taste 3.50 (typical tempeh). Based on the data, L2J1 has met SNI 3144:2015. Based on SNI 3144:2015, tempeh has a uniform white color on the entire surface of the tempeh, has a distinctive tempeh aroma without any ammonia odor, and has compact mycelium, when sliced it remains intact (does not fall off easily) [37].

3.5. IC_{50} Antioxidant Activity

The IC_{50} antioxidant activity is the concentration needed to inhibit free radicals by up to 50%. The IC_{50} value is a reference for determining the ability to inhibit free radicals. The smaller the IC_{50} value, the higher or stronger the antioxidant activity [29]. In this study, the IC_{50} antioxidant activity test was carried out on germinated jack bean tempeh with the best treatment, namely tempeh with a fermentation period of 60 hours packaged using plastic (L2J1). L2J1 has an IC_{50} value of 6,951.38 ppm, so its antioxidant activity is included in the weak category (>150 ppm) [53].

The IC_{50} antioxidant activity of germinated jack bean tempeh is likely influenced by the phenolic content which is a type of antioxidant compound. Phenolic content has a significant relationship with the IC_{50} value but with a negative relationship. The higher the phenolic content, the lower the IC_{50} value, but the higher or stronger the antioxidant activity [29]. The phenolic content of L2J1 is 0.97 mg GAE/g which is included in the low category (<10 mg GAE/g) [52]. Therefore, L2J1 has a high IC_{50} value, but its antioxidant activity is weak.

4. CONCLUSION

Different fermentation times significantly affect the phenolic content and hedonic quality of germinated jack bean tempeh, but the type of packaging does not significantly affect it. The interaction between fermentation time and packaging type significantly affected the phenolic content and hedonic quality of color, aroma and appearance of germinated bean jack bean tempeh. The optimal combination was found with a fermentation period of 60 hours, using plastic packaging, which caused the best results in terms of phenolic content and quality. The tempeh has a phenolic content that is included in the low category and the IC_{50} value shows a number >150 ppm, so its antioxidant activity is included in the weak category. It has a white colour, no beany aroma, a compact appearance, and a typical tempeh texture taste. Based on its phenolic content and antioxidant activity, the tempeh is not strong enough to inhibit oxidative stress. It is necessary to conduct studies on other functional properties, such as fiber, protein, or other antioxidant

compounds so that it can represent the potential of germinated jack bean tempeh as an antidiabetic functional food.

AUTHOR CONTRIBUTION

Friska Citra Agustia: Writing (review & editing), supervision, conceptualization, funding acquisition. **Rafida Salma**: writing (original draft), formal analysis. **Salma Azra Hamidah**: writing (original draft), formal analysis. **Kifayati Rosiyanti Dewi**: writing (review & editing), supervision. **Sanaz Sanayei**: writing (review & editing).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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