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Physicochemical and Antioxidant Properties of Germinated Soybean Tempe after Two Days Additional Fermentation Time

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Abstract: Tempe contains antioxidant components, which will increase if the soybean is germinated. Tempe is usually consumed in the fresh form two days after the fermentation process, even though its antioxidant level is higher after extending for 2 days. This study aimed to evaluate the changes in physicochemical and antioxidant properties of germinated soy tempe due to 2-days additional fermentation time. The six samples from germination (germinated and non-germinated soybean) and additional fermentation time (0, 1, and 2 days) treatments were analyzed for their physicochemical (sensory, texture, color, acidity, proximate content) and antioxidant components (antioxidant capacity, total free phenolic compound, and isoflavones). The results showed the decline in sensory properties of tempe due to additional fermentation time, but this food was still worth eating, without any "dislike" score overall. The decline was linear with the decrease in texture and increase in pH. The proximate content, color, and water activity experienced no changes. Meanwhile, antioxidant components increased during the additional fermentation time. The effects of soybean germination on antioxidant components were not dominant, except for isoflavones. In conclusion, the additional fermentation time only slightly decreased the sensory quality without changing the proximate composition with an increase in the antioxidant properties.

Keywords: isoflavones; over-fermented; phenolic compound; texture analyzer.

1. Introduction

Indonesian soybean fermented food contains free isoflavones, namely daidzein, and genistein [1–6]. Both isoflavones are classified as free phenolic compounds with better antioxidant activity than their bound form [7,8]. The functional aspect of tempe is improved by increasing the soybean quality through the germination process. This increases its products' antioxidant capacity, free phenolic compounds, daidzein, and genistein [2,9]. With these antioxidant properties, tempe is suspected to be able to tackle some diseases such as diabetes, oxidative stress, osteoporosis, and cardiovascular [10–15].

Generally, people consume tempe immediately after the fermentation process because the quality depreciates, assuming it is consumed a day or 2 after ripening [16,17]. However, this type of tempe is suspected of having higher functional components due to the additional fermentation time. In addition, the increase of antioxidant components such as free phenolic

compounds and isoflavones in overripe ones was reported in prior studies [18–20]. However, the relationship between the decrease and increase in physicochemical quality and antioxidant constituents, respectively, due to the combination of soybean germination and additional fermentation time has never been investigated.

This study aimed to evaluate the changes in physicochemical and antioxidant properties of tempe by combining soybean germination and additional fermentation time. Furthermore, six types of tempe were used as the samples, comprising both germinated and non-germinated soybeans fermented for 2, 3, and 4 days (0, 1, and 2 days of additional time).

2. Materials and Methods

2.1. Materials.

The materials used for tempe production were imported Genetically Modified Organisms (GMO) soybean (Sb&B Food Inc., Casselton, USA) and commercial tempe mold Raprima^{TM} (Bandung, Indonesia). Conversely, the material used for analysis consisted of reagents for the proximate test, isoflavones (daidzein and genistein), methanol, ammonium acetate, and 37% HCl from Merck (Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu, and gallic acid from Sigma-Aldrich (USA).

2.2. Tempe production and sample preparation.

Germinated soybeans were obtained from the soybean germination process-based proposed method explained by Astawan *et al.* [20]. The soybeans were sorted, soaked for 2 hours, rinsed, and stored in a dark place for 28 hours. Furthermore, they were watered every 3 hours till radicules of 2 to 5 mm long sprouted. These soybeans are called germinated soybeans.

The tempe production process referred to the method Astawan et al. [21] proposed. The germinated and non-germinated soybean were processed by boiling, soaking for 18 hours, peeling, washing, rinsing with hot water, air-drying, and inoculating with tempe molds (1 g mold/kg dry soybean). Afterward, it was covered with perforated plastic and incubated for 2, 3, and 4 days at a temperature of 30°C. Some of the tempe was tested for its physicochemical properties, including hedonic sensory test, color, texture, pH, titratable acidity, and water activity. The remaining were floured and had the fat removed for antioxidant-related analysis, including antioxidant capacity, total free phenolic content, daidzein, and genistein.

The tempe was floured by adopting the method proposed by Astawan *et al.* [22], where it was cut into slices of 1 cm, steamed for 10 minutes, dried, milled, and sieved with a 60-mesh sieve. Furthermore, fat was removed according to a modified method designed by Puteri *et al.* [23] by extracting the flour in hexane (1:3 w/v), stirring for 1 hour, and filtering; the entire process was repeated twice. The process was continued by re-extraction in hexane (1:3 w/v), sonicated for 20 minutes, filtered, dried in an oven at 50°C for 2 hours, and stored at 4°C. The flour was then used for isoflavones (daidzein and genistein), total free phenolic content, and antioxidant capacity analysis.

2.3. Sensory analysis.

The analysis was a hedonic test performed according to the Lawless and Heymann method [24], by employing 70 naive panelists to assess tempe with scales 1 = highly dislike, 2 = dislike, 3 = slightly dislike, 4 = neutral, 5 = slightly like, 6 = like, and 7 = highly like. The

sample dimensions were 1 x 2 x 5 cm in both uncooked (for color, aroma, texture, and overall attributes assessment) and fried forms (for taste attributes assessment). Meanwhile, both samples were served monadically and neutralized with drinking water.

2.4. Color analysis.

Colour analysis was performed using a chromameter instrument (Minolta CR-300, US) after cutting the whole samples, leaving the middle part to be tested. The device was then calibrated before analyzing using a white plate. The measurement was performed by shooting the device on a flat surface, with output recorded as L, a, and b scale values.

2.5. Texture analysis.

This analysis was performed using a texture analyzer TA-XT2i instrument (Stable Microsystem, UK) [25]. Tempe samples that are 35 cm thick were analyzed with a TA-43 (knife-like) probe at a rate of 1.32 cm/s. The graph's peak and area under the curve were then reported as the hardness and slicing power, respectively.

2.6. Proximate analysis.

The proximate analysis was performed according to the method proposed by AOAC [26], which consisted of water, ash, protein, fat, and carbohydrate contents (by difference) evaluation.

2.7. Water activity measurement.

The water activity was measured using a water activity meter instrument (Amittari WA 360A, Guangzhou, China). As much as 1 gram of tempe was weighed and inserted in a container to be tested.

2.8. Measurement of pH.

This measurement was carried out using a pH meter (Eutech 700, USA) [27]. Before the test, the buffer solution with pH 4 and 7 was used to calibrate the instrument. Furthermore, 10 grams of tempe were mashed in a mortar, and water was slowly added (1:2 w/v). Afterward, the pH of the sample was measured.

2.9. Titratable acidity.

The titratable acidity (TA) test was carried out in accordance with a similar method formulated by Nurdini *et al.* [27]. First, a mixture of 1 g (Ws) mashed tempe and 50 mL of water was filtered, then 10 ml of the filtrate was added to a phenolphthalein indicator. The sample was titrated with standardized 0.1 N NaOH until the color changed from colorless to pink. The TA value was reported as g eq lactic acid/100 g using the following equation.

$$\text{g eq lactic acid/100 g} = \frac{\text{V NaOH (mL) x N NaOH (mol/L) x MW of lactic acid (g/mol)x Dillution x 100}}{\text{Ws (g) x valence lactic acid x 1000 } (^{\text{mL}}/_{\text{L}})}$$

2.10. Analysis of antioxidant capacity.

The analysis of antioxidant capacity was performed using DPPH inhibition assay1 (1,1-diphenyl-2-picrylhydrazyl) with a modified extraction sample [28]. A gram of free fat tempe flour and 10 mL of methanol and water with a ratio of 80 : 20 mixture was vortexed for 1 minute and centrifuged at 4°C, 3000 rpm speed, for 45 minutes. A 0.2 mL of supernatant samples and ascorbic acid (25 to 200 µg/mL) were each added with 3.8 mL of 0.1 mM DPPH (in methanol), incubated in a dark place for 30 minutes, and its absorbance (A_1) was measured at 517 nm wavelength. A blank sample without sample additions was also measured to determine its absorbance (A_0). The tempe antioxidant capacity was obtained by comparing sample inhibition and ascorbic acid percentage using the following formula.

$$\%\text{inhibition}=\frac{A_0\text{-}A_1}{A_0}\times 100$$

2.11. Total free phenolic content analysis.

The test was carried out based on the method formulated by Chen *et al.* [28], using a similar extraction step as antioxidant capacity analysis. Supernatant samples with a volume of 0.1 mL resulted from the aforementioned process and gallic acid standard (50 to 250 µg/mL) were each added with 2 mL of 2% Na_2CO_3 and settled for 3 minutes. Afterward, 0.1 mL of 50% Folin-Ciocalteu was added and incubated for 30 minutes. The total free phenolic content was obtained by comparing the sample and gallic acid standard at 750 nm wavelength.

2.12. Isoflavone analysis.

Isoflavone analysis was performed using the method proposed by Astawan *et al.* [2]. A mixture of 2 grams of defatted tempe flour and 30 mL of 1N HCl and acetonitrile with a ratio of 1 : 4 was stirred for 2 hours, filtered, and the filtrate obtained. This was diluted 10-folds with a mixed mobile phase of methanol and ammonium acetate 1 µM with a ratio of 3 : 2. Daidzein and genistein standards and sample solution (0.5 to 50 µg/mL) were filtered using a nylon filter membrane syringe of 0.22 µm. In addition, 20 µL of the solution was injected into an HPLC (high-performance liquid chromatography) instrument (Agilent 1200 Series, Agilent Technologies, USA). The analysis was carried out using C-18 5 µm (15 cm x i.d. 4.6 mm) column at room temperature with a flow rate of 0.5 mL/minute and an isocratic pump system with a multiwavelength (MW) detector at 265 nm wavelength.

2.13. Data analysis.

The data were processed statistically using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at a 95% confidence interval using SPSS 22 software.

3. Results

3.1. Physicochemical properties.

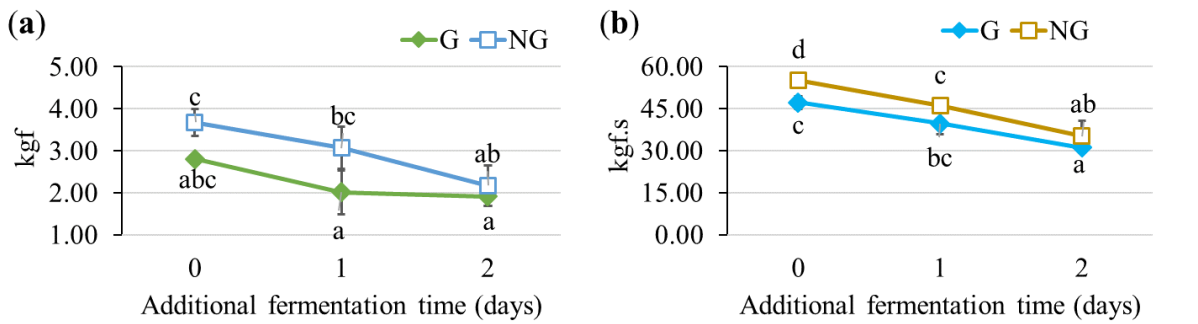
The color of tempe was determined with a chromameter and reported as L, a, b, and h* values. Meanwhile, the L scale indicates brightness, a scale indicates red (positive) or green (negative) color intensities, the b scale indicates yellow (positive) or blue (negative) color intensities, and h* is the hue angle [29]. The analysis result showed that the germination process

and additional fermentation time had an insignificant effect on the product color (Table 1). L scale (71.43 – 73.39), a (1.52– 2.53), b (18.23 – 20.95), and h* (82.27 – 85.58) values showed that the product had a dominant yellow color with a slight hint of red and high brightness level [29].

According to the texture analysis, there was a decrease in tempeh's hardness and slicing power due to soybean germination and additional fermentation time of up to 2 days (Figures 1a and 1b). Furthermore, the hardness point shifted inward along with the additional fermentation period (Figure 2a -2f). The graph peak shifted over time (to the right).

Table 1. The value of L, a, b, and h* in the Hunter scale.

Tempe	L	a	b	h*
G0	72.45±3.45	1.98±0.40	19.24±1.91	84.15±0.72
NG0	72.92±2.08	1.71±0.30	19.46±1.53	84.94±1.06
G1	71.71±5.09	1,52±0,46	19.41±2.63	85.58±0.70
NG1	73.39±1.70	1.84±0.48	20.95±2.05	84.88±1.67
G2	71.43±5.99	2.53±0.94	18.23±2.59	82.27±1.77
NG2	73.20±1.65	1.80±0.23	19.30±1.36	84.65±0.74



G = with germinated soybean; NG = with non-germinated; 0, 1, and 2 = additional fermentation time (days). Not any significantly different ($p>0.05$) value (mean ± SD, n = 8) was found among all the samples on the same scale.

Figure 1. The texture of tempe reported as: (**a**) hardness, (**b**) slicing power. G = with germinated soybean; NG = with non-germinated soybean; 0, 1, and 2 = additional fermentation time (days). The various superscripts showed a significantly different ($p<0.05$) value (mean ± SD, n = 6) when analyzed by DMRT posthoc test.

Based on the sensory test result, the hedonic scores of color, aroma, taste, texture, and overall attributes decreased due to an additional fermentation time of relatively 2 days, either in germinated or non-germinated soy tempe (Figure 3). Despite the decline, all samples had the least neutral score (4), except G2 tempe. The overall hedonic score of G0 was not significantly different from NG0. Besides, this result was aligned with the color, aroma, and taste. On the contrary, each NG1 and NG2 had higher overall hedonic scores compared to G1 and G2, respectively. However, their taste and texture were not significantly different ($p>0.05$), and the decrease in hedonic value aligned with the hardness and slicing power values (Figure 1a-1b).

The tempe proximate contents (except water) (Table 2) and water activity (Figure 4a and 4b) were not significantly different ($p>0.05$) due to the germination process and additional fermentation of relatively 2 days. The water content of germinated soy tempe (Figure 4a) decreased within the 2-days additional fermentation time, while the non-germinated sample increased after a day and decreased again after 2 days. It was discovered that the germinated soy tempe had higher water content compared to the non-germinated sample.

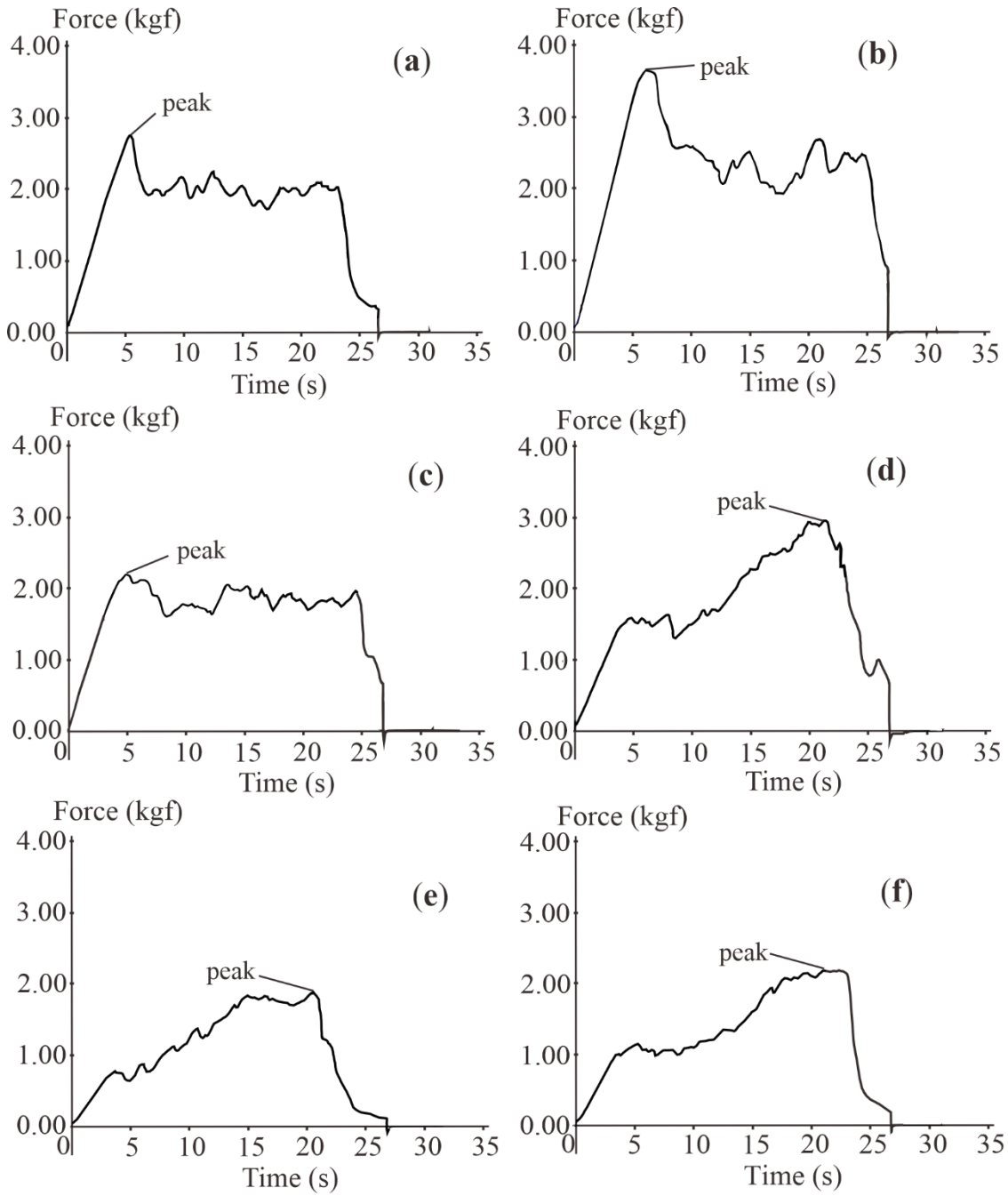


Figure 2. Output graph of texture analysis (**a-f** = G0, NG0, G1, NG1, G2, NG2) G = with germinated soybean; NG = with non-germinated soybean; 0, 1, and 2 = additional fermentation time (days).

Tempe acidity was determined based on pH value and titratable acidity (TA) parameters (Figure 5). The pH value increased during the 2-days additional fermentation time. The soybean germination process only affected the pH of tempe when no additional fermentation time was applied. The pH and TA values of G0 were lower and higher than NG0, respectively. The TA of germinated soy tempe increased after a day and further decreased after 2-days. In addition, the TA of non-germinated soy tempe increased after a day and then became stable after 2-days.

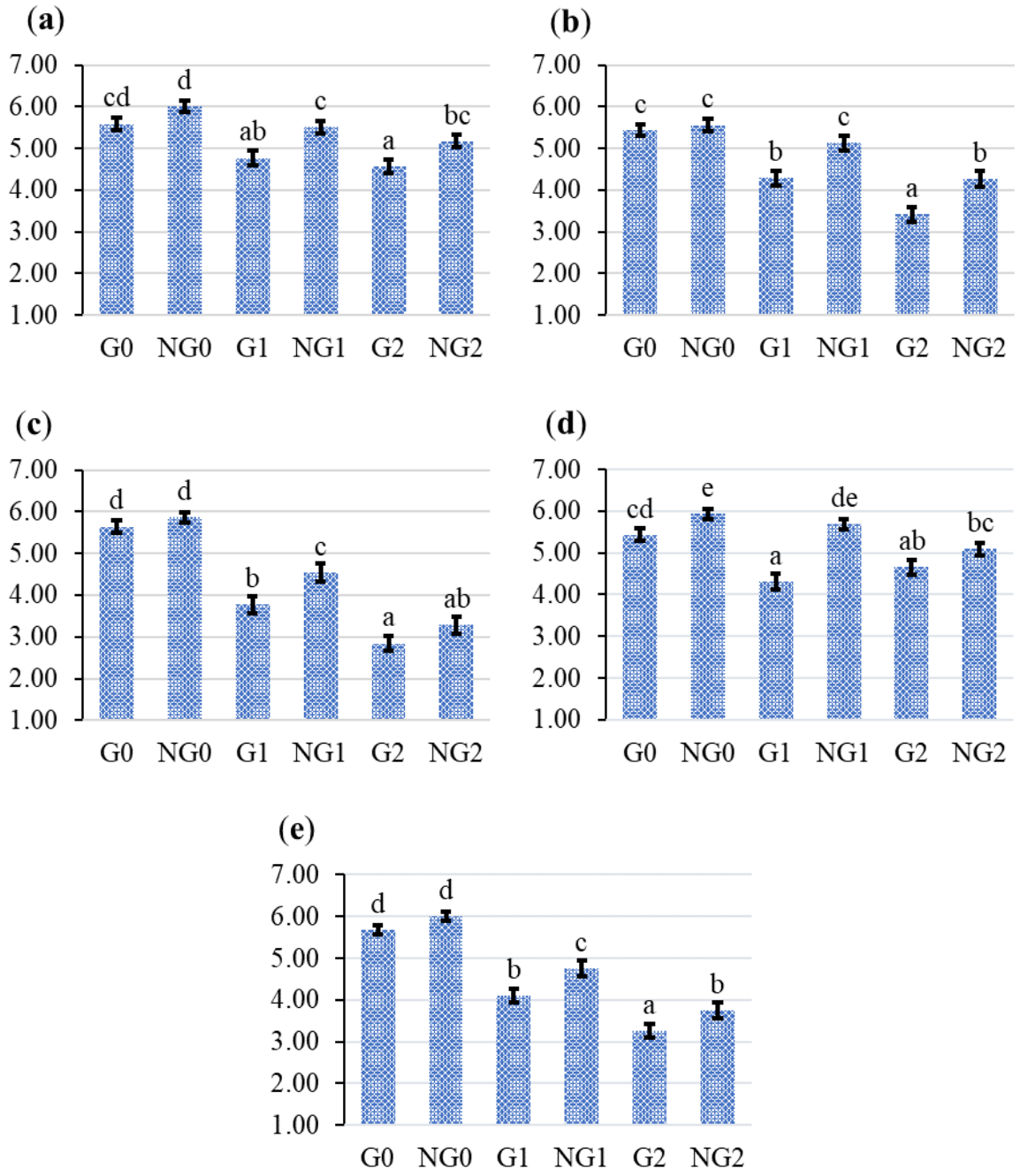


Figure 3. The hedonic score of (**a**), color ; (**b**) aroma ; (**c**) taste; (**d**) texture and overall (**e**) in tempe. G = with germinated soybean; NG = with non-germinated soybean; 0, 1, and 2 = additional fermentation time (days). Various letters showed a significant ($p<0,05$) different value (mean ± SE; n = 70) analyzed by DMRT posthoc test .

3.2. Antioxidant properties.

All parameters related to antioxidants increased during the 2-days additional fermentation time (Table 3). This includes a total free phenolic content, antioxidant capacity, daidzein, and genistein. The soybean germination process significantly increased daidzein and genistein in tempe without additional fermentation. However, after this period, the germination process caused a decrease in daidzein and genistein content in tempe. In accordance with the antioxidant capacity and total free phenolic content parameters, the effect of soybean germination was only detected after a day. There was an increase in the antioxidant capacity and a decrease in the total free phenolic content.

Table 2. Non-water proximate content of tempe.

Tempe	Ash (%db)	Fat (%db)	Protein (%db)	Carbohydrate (%db)
G0	2.33±0.01	15.18±3.69	50.04±3.31	32.44±2.98
NG0	2.38±0.01	12.87±2.03	46.89±3.65	37.87±3.22
G1	2.18±0.01	14.83±3.07	52.00±4.61	30.98±7.21
NG1	2.49±0.07	14.54±2.90	51.87±1.10	31.09±1.17
G2	2.17±0.18	16.59±6.21	48.87±4.97	32.37±2.43
NG2	2.20±0.16	18.28±6.87	47.56±3.52	31.96±1.82

G = with germinated soybean; NG = with non-germinated soybean; 0, 1, and 2 = additional fermented time (days); db = dry based. Carbohydrate content was analyzed with the "by difference" method. The values (mean ± SD, n = 2) in the same parameter showed an insignificantly different value (p>0.05).

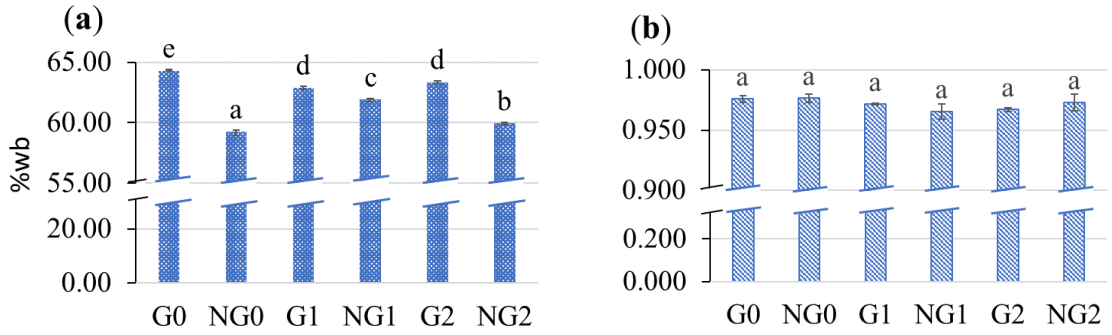


Figure 4. (**a**) Water content and (**b**) water activity of tempe. G = with germinated soybean; NG = with non-germinated soybean; 0, 1, and 2 = additional fermentation time (days); wb = wet basis. The values (mean ± SD, n = 2) with various letters showed a significantly different value (p<0.05) analyzed by DMRT posthoc test.

4. Discussion

4.1. Changes in physicochemical properties.

The changes in physicochemical properties varies due to the germination process and additional fermentation time. Fortunately, the soybean germination procedure increased the water content (Figure 4a), according to preliminary studies [30]. This was caused by the soaking and periodic watering processes that led to the excessive absorption of water in the soybean matrix [31]. In addition, the legumes' germination procedure also increased the simple hydrophobic compounds, which led to the absorption of more water soybean matrix [32].

The combination of the soybean germination process and additional fermentation time decreased the hardness and slicing power of the tempe texture and shifted its point inward (Figures 1 and 2). This decline was initially reported before in tempe with 1-day additional fermentation time [25]. The observation carried out with Scanning Electron Microscope (SEM) showed an increased irregularity in soybean cell wall structure along with the additional time [25]. Meanwhile, changes in tempe texture due to the germination process have never been reported. However, a decrease in bread [33], chips [34], and yogurt [35] made from germinated soy flour has been reported.

Increased hydrolyzing enzymes caused the decrease in tempe hardness and slicing power due to certain treatments during the germination process and additional fermentation time. The increased enzyme activity increased the hydrolysis rate and degradation of the macromolecule components, including the soybean cell walls [25]. Besides, the increase in water content also produced a softer texture [32].

The decrease in hardness and slicing power caused a decline in the textural hedonic score (Figure 3d). This result showed that the preferred tempe had a more rigid texture, while those with a softer texture were caused by a combination of the soybean germination process

and additional fermentation time. The treatment made the texture softer, leading to a lower consumer preference. Aside from texture, the hedonic scores of color, taste, aroma, and overall attributes also decreased.

According to the panelists' comments (data not shown), tempe with a low hedonic color is darker due to the application of both treatments. The previous study also reported several reasons for these changes [36], such as more molds experiencing the death phase, the presence of oxidized double chain fatty acids (such as linoleic and linolenic acids), and *Klebsiella pneumonia* which produces dark red vitamin B12.

The chromameter instrument did not detect significant differences in tempe hedonic color (Figure 3a) due to these treatments (Table 1). This result was possibly caused by the color specificity tested by the chromameter, which only measured a point on the sample's surface [37]. Tempe has various colors on the surface, and when measured using a chromameter, the differences are unidentifiable. Besides, the sensory observation performed by the panelists using their eyes showed no difference was detected among the samples.

Based on the panelists' comments (data not shown), the decline in taste and aroma hedonic scores (Figure 3b-3c) were related to the slightly acidic and bitter taste and an ammonia odor that emanated during the additional fermentation time. The decreased score was parallel to the increased pH value (Figure 5) due to the formation of ammonia compounds in gaseous and ionic forms during fermentation [38]. In addition, ammonia gas affects tempe aroma, while ammonium ion has an alkaline pH and impacts the taste [39–41]. This is consistent with the results of similar preliminary studies [25,27,36,42].

Subsequently, the taste and aroma score decrease during the fermentation process was also caused by protein hydrolysis into peptides and amino acids. Both compounds are known to contribute to food taste, such as umami, sourness, and bitterness [43]. This was suspiciously the reason for the lesser taste score of germinated soy tempe compared to the non-germinated seedlings during additional fermentation time. The germination process triggered the protein hydrolysis intensity and produced more peptides and amino acids, and similar sensory results were also discovered in other soy-derived products [33,35].

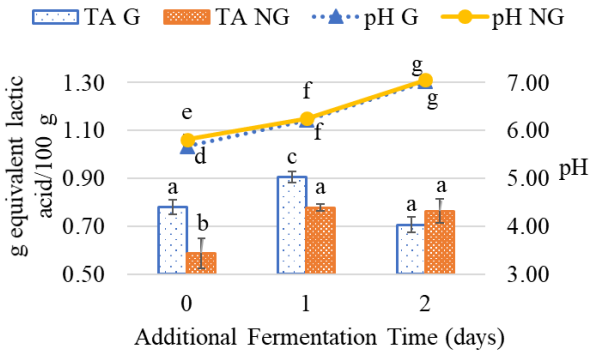


Figure 5. Titratable acidity (TA) and pH value of tempe. G = with germinated soybean; NG = with non-germinated. The various letters showed significantly ($p<0.05$) different values (mean ± SD, n = 2) analyzed by DMRT posthoc test.

The effect of germination on pH was only observed on tempe with no additional fermentation time. Subsequently, the pH value of germinated soy tempe was lower compared to the non-germinated sample. This was caused by the excessive organic acids found in germinated soy tempe, as is evident in the titratable acidity result. Preliminary studies have been carried out on soy milk [44] and fermented probiotic drinks [45].

Due to additional fermentation time, the change in titratable acidity (TA) value does not align with the pH value. The increase in tempe due to a day of additional fermentation time has been reported in preliminary studies [27,46], but not for 2-days. This is in line with the result of this study, which stated the increase in TA was caused by the hydrolysis of macro components and the microbe metabolism output, which increased the organic acids. This includes lactic [47], amino [8], and fatty acids [46]. Meanwhile, hydrolysis during the germination process also increased the organic acid content in the soybean [48,49].

In the 2-days additional fermentation time, there was a decrease in the TA of germinated soy tempe, while the non-germinated was stagnant. Besides, the mechanism of this phenomenon is still unknown. However, the stable TA was suspiciously caused by the presence of excess ammonia during the fermentation [38]. This caused an increase and decrease in pH and dissociated organic acids, respectively. However, further studies need to be carried out to confirm this situation.

The overall hedonic score of tempe experienced a decline due to changes in physicochemical properties. However, the lowest score was relatively 4 (neutral), except for G2. All hedonic scores of the samples did not reach 2 (dislike), which showed that the product was still consumable. Many panelists also scored tempe with an additional fermentation time of relatively 2-days 7 (like), because it is widely used as a food seasoning. However, the consumption method is dissimilar to the fresh one [43].

Besides the changes in the above-mentioned physicochemical parameters, the proximate tempe compositions of the sample, except water, remained the same, as shown in Table 2. This is different from Astawan *et al.*'s [30], which stated that germinated soy tempe had higher protein and lower fat content. However, Puspitasari *et al.* reported no changes in proximate compositions except water in the germinated soybean, which aligns with this study. These different results were assumed to be caused by the raw material or soybean species. A study by Astawan *et al.* [30] used Grobogan local soybean, while Puspitasari *et al.* [9] utilized imported non-GMO soybean. This study employed imported non-GMO soybean. Therefore, the tempe had similar results, which aligned with Puspitasari *et al*. It can be deduced that the soybean type probably affects the proximate composition patterns.

Table 3. Antioxidant properties of tempe in dry weight.

Tempe	Antioxidant capacity (mg AEAC/100 g)	Total free phenolic (mg GAE/100 g)	Daidzein (mg/100 g)	Genistein (mg/100 g)
G0	30.30±0.05^{a}	90.79±3.25^{a}	50.03±1.81^{b}	110.27±4.47^{b}
NG0	27.30±2.44^{a}	85.53±1.61^{a}	44.98±1.89^{a}	101.00±4.66^{a}
G1	45.22±1.28^{c}	116.13±4.89^{b}	54.28±1.45^{b}	119.82±2.80^{c}
NG1	41.51±2.86^{b}	128.44±7.39^{c}	60.93±1.42^{c}	133.60±1.77^{d}
G2	51.34±0.61^{d}	132.56±1.19^{cd}	62.12±1.31^{c}	136.01±2.52^{d}
NG2	51.02±1.12^{d}	138.52±6.53^{d}	74.43±2.79^{d}	161.18±2.34^{e}

0, 1, and 2 = additional fermentation time (days); G = using germinated soybean; NG = using germinated soybean. GAE = gallic acid equivalent; AEAC = ascorbic acid equivalent antioxidant capacity. The values (mean ± SD, n = 3) of various superscripts showed significantly different values analyzed by DMRT posthoc test.

The water activity showed no differences from both treatments (Figure 4b). This result differed from the water content, which was lower in non-germinated soybean because it has not reached the level that affects the water activity. According to these results, water that changed in tempe was in types-3 and 4, free and unbounded water in food material [50].

4.2. The increase of antioxidant properties.

The antioxidant characteristics in tempe increased due to an additional fermentation time of relatively 2 days (Table 3). The increase in total free phenolic content, daidzein, and genistein is in line with previous studies with variations of 1 to 5 days [1,5,18,51,52]. This increase is caused by the breakdown of phenolic by carbohydrate-hydrolyzing enzymes from the microbes, such as α-/β-glucosidase, α-amylase, and β-glucuronidase [53–56]. The daidzein and genistein isoflavones, classified as free phenolic compounds, were triggered due to the β-glucosidase activity [4,57]. Therefore, this caused an increase in antioxidant capacity in accordance with the additional fermentation time.

The effect of soybean germination was dominant in daidzein and genistein; however, it was insignificant in the total free phenolic content and antioxidant capacity. The impact was evident in the additional fermentation time of approximately 1 day in both parameters. Furthermore, its effect on antioxidant capacity was insignificant compared to the additional fermentation time. This was probably due to the contribution of other components except for phenolic compounds, such as peptides [58,59].

The germination process increased the total free phenolic contents, namely daidzein and genistein in soybean [60,61], and its application as a raw material also showed a similar result [2]. However, when combined with additional fermentation time, the isoflavone contents in the germinated soybean were lower compared to the non-germinated sample [9]. Besides, a similar result was obtained in this study, although the definite cause of this phenomenon is still unknown. The hydrolysis rate was probably higher in the non-germinated sample, which was detected during the additional fermentation time. This notion is based on the increase of antioxidant components in non-germinated soy tempe compared to the germinated sample during the 2-days additional fermentation time.

5. Conclusions

Physicochemically, soybean germination, and additional fermentation time treatments resulted in tempe with a lower acceptance value than common tempe (non-germinated soy tempe with no additional fermentation time). This was proven by the decreasing hedonic scores and supported by objective test results, such as texture, pH, titratable acidity, and water content. However, the decline in overall hedonic score was still in the "neutral" scale value.

Nutritionally, the proximate composition in tempe did not change, except for its water content. However, when observed from the antioxidant component, the additional fermentation time produced higher antioxidant capacity, total free phenolic content, daidzein, and genistein. The germination process does not contribute to the additional fermentation time. Hence, non-germinated soy tempe with an additional 2-days is considered a high potential functional food because of its excessive antioxidant properties and acceptable sensory quality.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Hashim, N.; Tai, C.W.; Wen, H.X.; Ismail, A.; Kong, K.W. Comparative Evaluation of Antioxidant Properties and Isoflavones of Tempe Fermented in Two Different Wrapping Materials. *Curr. Res. Nutr. Food Sci.* **2018**, *6*, 307–317, https://doi.org/10.12944/CRNFSJ.6.2.06.
2. Astawan, M.; Rahmawati, I.S.; Cahyani, A.P.; Wresdiyati, T.; Putri, S.P.; Fukusaki, E. Comparison Between the Potential of Tempe Flour Made from Germinated and Nongerminated Soybeans in Preventing Diabetes Mellitus. *HAYATI Journal of Biosciences* **2020**, *27*, 16–23, https://doi.org/10.4308/hjb.27.1.16.
3. Huang, Y.C.; Wu, B.H.; Chu, Y.L.; Chang, W.C.; Wu, M.C. Effects of Tempe Fermentation with Lactobacillus Plantarum and Rhizopus oligosporus on Streptozotocin-Induced Type II Diabetes Mellitus in Rats. *Nutrients* **2018**, *10*, 1143, https://doi.org/10.3390/nu10091143.
4. Lo, D.; Rawendra, R.D.S.; Huang, C.S.; Khatri-Chhetri, R.; Wang, Y.T.; Wu, M.C. Anti-oxidative and protective effect of soy tempe on di(2-ethylhexyl)-phthalate (DEHP) injured FL83B mice liver cells. *IOP Conf. Ser.:Earth Environ. Sci*. **2018**, *195*, 1-8, https://doi.org/10.1088/1755-1315/195/1/012058.
5. Tsuji, H.; Nozaki, N.; Nakajima, N.; Ishihara, K.; Ishikawa, A. Analysis of isoflavone content in tempe, a fermented soybean, and preparation of a new isoflavone-enriched tempe. *Journal of Bioscience and Bioeng*innering **2005**, *100*, 685–687, https://doi.org/10.1263/jbb.100.685.
6. Ahnan-Winarno, A.D.; Cordeiro, L.; Winarno, F.G.; Gibbons, J.; Xiao, H. Tempe: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*,1717–1767, https://doi.org/10.1111/1541-4337.12710.
7. Krizova, L.; Dadakova, K.; Kasparovska, M.; Kasparovsky, T. Isoflavones. *Molecules* **2019**, *24*, 1076, https://doi.org/10.3390/molecules24061076.
8. Ali, N.M.; Yeap, S.K.; Yusof, H.M.; Beh, B.K.; Ho, W.Y.; Koh, S.P.; Abdullah, M.P.; Alitheen, N.B., Long, K. Comparison of free amino acids, antioxidants, soluble phenolic acids, cytotoxicity and immunomodulation of fermented mung bean and soybean. *Journal of the Science of Food and Agriculture* **2016**, *96*, 1648–1658, https://doi.org/10.1002/jsfa.7267.
9. Puspitasari, A.; Astawan, M.; Wresdiyati, T. The Effect of soybeans germination on proximate composition and isoflavones bioactive components of fresh and semangit tempe. *Pangan* **2020**, *96*, 35–44, https://doi.org/10.33964/jp.v29i1.460.
10. Chen, Y.C.; Tao, N.L.; Hu, S.Y.; Tsai, H.Y.; Liao, S.C.; Tsai, W.L.; Hu, C.Y. Effect of Tempe on Gut Microbiota and Anti-Stress Activity in Zebrafish. *Int. J. Mol. Sci*. **2021**, *22*, 12660, https://doi.org/10.3390/ijms222312660.
11. Su, H.K.; Tsai, M.H.; Chao, H.R.; Wu, M.L.; Lu, J.H. Data on effect of Tempe Fermentation on patients with type II diabetes. *Data Br*. **2021**, *38*, 107310, https://doi.org/10.1016/j.dib.2021.107310.
12. Gofur, A.; Witjoro, A.; Arifah, S.N.; Atho'illah, M.F.; Annisa, Y.; Lestari, S.R. Black soybean tempe and purple sweet potato improve sperm quality in streptozotocin-induced diabetic rats. *Veterinary World*. **2020**, *13*, 2534–2540, https://doi.org/10.14202/vetworld.2020.2534-2540.
13. Romulo, A.; Surya, R. Tempe: A traditional fermented food of Indonesia and its health benefits. *International Journal of Gastronomy and Food Science* **2021**, *26*, 100413, https://doi.org/10.1016/j.ijgfs.2021.100413.
14. Surya, R.; Romulo, A.; Suryani, Y. Tempe extract reduces cellular ROS levels and upregulates the expression of antioxidant enzymes. *Food Research*, **2021**, *5*, 121–128, https://doi.org/10.26656/fr.2017.5(3).560.
15. Wresdiyati, T.; Firdaus, A.; Astawan, M. Tempe and Soybean var. Grobogan-Indonesia Increased the Number of Osteoblasts and Osteocytes, Inhibited Osteoclast Damage in the Tibia Bone of Rats. *HAYATI Journal of Biosciences* **2021**, *28*, 144, https://doi.org/10.4308/hjb.28.2.144.
16. Astawan, M.; Hermanianto, J.; Suliantari; Sugiyanto, G.S.P. Application of vacuum packaging to extend the shelf life of fresh-seasoned tempe. *I.F.R.J*. **2016**, *23*, 2571–2580.
17. Aaslyng, M.D.; Højer, R. Introducing Tempe as a New Plant-Based Protein Food Item on the Danish Market. *Foods* **2021**, *10*, 2865, https://doi.org/10.3390/foods10112865.
18. Kuligowski, M.; Pawłowska, K.; Jasińska-Kuligowska, I., Nowak, J. Isoflavone composition , polyphenols content and antioxidative activity of soybean seeds during tempe fermentation. *CyTA – Journal of Food* **2017**, *15*, 27–33, http://doi.org/10.1080/19476337.2016.1197316.
19. Chang, C.T.; Hsu, C.K.; Chou, S.T.; Chen, Y.C.; Huang, F.S.; Chung, Y.C. Effect of fermentation time on

the antioxidant activities of tempe prepared from fermented soybean using *Rhizopus oligosporus*. *I.F.S.T.* **2009**, *44*, 799–806, http://doi.org/10.1111/j.1365-2621.2009.01907.x.
20. Astawan, M.; Wresdiyati, T.; Yoshari, R.M.; Rachmawati, N.A.; Fadilla, R. The Physicochemical Properties of Tempe Protein Isolated from Germinated and Non-Germinated Soybeans. *Journal of Nutritional Science and Vitaminology* **2020**, *66*, S215–S221, http://doi.org/10.3177/jnsv.66.S215.
21. Astawan, M.; Wresdiyati, T.; Subarna; Rokaesih; Yoshari, R.M. Functional properties of tempe protein isolates derived from germinated and non-germinated soybeans. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *443*, https://doi.org/10.1088/1755-1315/443/1/012001.
22. Astawan, M.; Wresdiyati, T.; Purnomo, E.H.; Purwanto, A. Equivalence Test Between the Physicochemical Properties of Transgenic and Non-Transgenic Soy Flour. *Journal of Nutritional Science and Vitaminology* **2020**, *66*, S286–S294, http://doi.org/10.3177/jnsv.66.S286.
23. Puteri, N.E.; Astawan, M.; Palupi, N.S.; Wresdiyati, T.; Takagi, Y. Characterization of biochemical and functional properties of water-soluble tempe flour. *Food Sci. Technol.* **2018**, *38*, 147–153, http://doi.org/10.1590/fst.13017.
24. Lawless, H.T.; Heymann, H. Acceptance and Preference Testing. Sensory Evaluation of Food, **1999**, 430–479, http://doi.org/10.1007/978-1-4419-7452-5_13.
25. Handoyo, T.; Morita, N. Structural and Functional Properties of Fermented Soybean (Tempe) by Using *Rhizopus oligosporus*. *International Journal of Food Properties* **2006**, *9*, 347–355, https://doi.org/10.1080/10942910500224746.
26. Latimer, G.W. Official Method of Analysis of AOAC International. *AOAC International* **2019**, *3*.
27. Nurdini, A.L.; Nuraida, L.; Suwanto, A.; Suliantari. Microbial growth dynamics during tempe fermentation in two different home industries. *International Food Research Journal* **2015**, *22*, 1668–1674, https://www.researchgate.net/publication/282794514_Microbial_growth_dynamics_during_tempe_fermentation_in_two_different_home_industries.
28. Chen, H.H.; Chang, H.C.; Chen, Y.K.; Hung, C.L.; Lin, S.Y.; Chen, Y.S. An improved process for high nutrition of germinated brown rice production: Low-pressure plasma. *Food Chemistry* **2016**, *191*, 120–127, http://doi.org/10.1016/j.foodchem.2015.01.083.
29. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.J. Colour Measurement and Analysis in Fresh and Processed Foods: a Review. *Food and Bioprocess Technology* **2013**, *6*, 36–60, http://doi.org/10.1007/s11947-012-0867-9.
30. Astawan, M.; Wresdiyati, T.; Ichsan, M. Physicochemical characteristics of germinated soybean tempe flour. *J. Gizi dan Pangan*. **2016**, *11*, 35–42, https://journal.ipb.ac.id/index.php/jgizipangan/article/view/13167/9919.
31. Bueno, D.B.; da Silva Júnior, S.I.; Seriani Chiarotto, A.B.; Cardoso, T.M.; Neto, J.A.; Lopes dos Reis, G.C.; Gloria, M.B.A. The germination of soybeans increases the water-soluble components and could generate innovations in soy-based foods. *LWT - Food Science and Technology* **2020**, *117*, https://doi.org/10.1016/j.lwt.2019.108599.
32. Warle, B.M.; Riar, C.S.; Gaikwad, S.S.; Mane, V.A. Effect of Germination on Nutritional Quality of Soybean (*Glycine Max*). *IOSR Journal of Environmental Science. Toxicology and Food Technology* **2015**, *9*, 13–16, https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1070.905&rep=rep1&type=pdf
33. Shin, D.J.; Kim, W.; Kim, Y. Physicochemical and sensory properties of soy bread made with germinated, steamed, and roasted soy flour. *Food Chemistry* **2013**, *141*, 517–523, http://doi.org/10.1016/j.foodchem.2013.03.005.
34. Maetens, E.; Hettiarachchy, N.; Dewettinck, K.; Horax, R.; Moens, K.; Moseley, D.O. Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans. *LWT - Food Science and Technology* **2017**, *84*, 505–510, http://doi.org/10.1016/j.lwt.2017.06.020.
35. Yang, M.; Li, L. Physicochemical, Textural and Sensory Characteristics of Probiotic Soy Yogurt Prepared from Germinated Soybean. *Food Technology and Biotechnology* **2010**, *48*, 490–496, https://www.researchgate.net/publication/263162878_Physicochemical_Textural_and_Sensory_Characteristics_of_Probiotic_Soy_Yogurt_Prepared_from_Germinated_Soybean.
36. Muzdalifah, D.; Athaillah, Z.A.; Nugrahani, W.; Devi, A.F. Colour and pH changes of tempe during extended fermentation. *AIP Conference Proceedings* **2017**, *1803*, http://doi.org/10.1063/1.4973163.
37. Keskin, M.; Arslan, A.; Soysal, Y.; Sekerli, Y.E.; Celiktas, N. Feasibility of a chromameter and chemometric techniques to discriminate pure and mixed organic and conventional red pepper powders: A pilot study. *Journal of Food Processing Preservation* **2021**, http://doi.org/10.1111/jfpp.15846.
38. Sparringa, R.A.; Owens, J.D. Protein Utilization during Soybean Tempe Fermentation. *J. Agric. Food Chem.* **1999**, *47*, 4375–4378, http://doi.org/10.1021/jf981279u.
39. Murata, K.; Ikehata, H.; Miyamoto, T. Studies on the Nutritional Value of Tempe. *Journal of Food Science* **1967**, *32*, 580–586, https://doi.org/10.1111/j.1365-2621.1967.tb00837.x.
40. Ruiz-Teran, F.; Owens, J.D. Chemical and Enzymic Changes During the Fermentation of Bacteria-Free Soya Bean Tempe. *Journal of Science of Food and Agticulture* **1996**, *71*, 523–530, https://doi.org/10.1002/(SICI)1097-0010(199608)71:4%3C523::AID-JSFA613%3E3.0.CO;2-R.
41. Prameswari, H.A.; Nursiwi, A.; Zaman, M.Z.; Ishartani, D.; Sari, A.M. Changes in Chemical and Sensory Characteristics of Gunungkidul's Lamtoro (*Leucaena Leucocephala*) Tempe During Extended Fermentation.

IOP Conf. Ser. Earth Environ. Sci. **2021**, *828*, https://doi.org/10.1088/1755-1315/828/1/012001.
42. Paredes-Lopes, O.; Harry, G.I. Changes in Selected Chemical and Antinutritional Components During Tempe Preparation Using Fresh and Hardened Common Beans. *Journal of Food Science* **1989**, *54*, 968–970, https://doi.org/10.1111/j.1365-2621.1989.tb07923.x.
43. Utami, R.; Wijaya, C.H.; Lioe, H.N. Taste of Water-Soluble Extracts Obtained from Over-Fermented Tempe. *International Journal of Food Properties* **2016**, *19*, 2063–2073, http://doi.org/10.1080/10942912.2015.1104509.
44. Jiang, S.; Cai, W.; Xu, B. Food Quality Improvement of Soy Milk Made from Short-Time Germinated Soybeans. *Foods* **2013**, *2*, 198–212, http://doi.org/10.3390/foods2020198.
45. Chavan, M.; Gat, Y.; Harmalkar, M.; Waghmare, R. Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume. *LWT - Food Science and Technology* **2018**, *91*, 339–344, https://doi.org/10.1016/j.lwt.2018.01.070.
46. Wagenknecht, A.C.;Mattick, L.R; Hand, D.B.; Lewin, L.M.; Steinkraus, K.H. Changes in Soybean Lipids Tempe Fermentation. *Journal of Food Science* **1961**, *26*, 373–376, https://doi.org/10.1111/j.1365-2621.1961.tb00375.x.
47. Hassanein, T.R.; Prabawati, E.K.; Dewi, M.; Tirtaningtyas, P. Analysis of chemical and microbial change during storage of overripe tempe powder as seasoning material. *Int. J. Sci. Eng.* **2015**, *8*, 131–134.
48. Gu, E.J.; Kim, D.W.; Jang, G.J.; Song, S.H.; Lee, J.I.; Lee, S.B.; Kim, B.M; Cho, Y.; Lee, H.J.; Kim, K.J. Mass-based metabolomic analysis of soybean sprouts during germination. *Food Chemistry* **2017**, *217*, 311–319, http://doi.org/10.1016/j.foodchem.2016.08.113.
49. Sun, W.X.; Zhang, R.J.; Fan, J.; He, Y.; Mao, X.H. Comprehensive transformative profiling of nutritional and functional constituents during germination of soybean sprouts. *Journal of Food Measurement and Characterization* **2018**, *12*, 1295–1302, http://doi.org/10.1007/s11694-018-9743-2.
50. Rongtong, B.; Suwonsichon, T.; Ritthiruangdej, P.; Kasemsumran, S. Determination of water activity, total soluble solids and moisture , sucrose, glucose and fructose contents in osmotically dehydrated papaya using near-infrared spectroscopy. *Agriculture and Natural Resources* **2018**, *52*, 557–564, https://doi.org/10.1016/j.anres.2018.11.023.
51. Ferreira, M.P.; de Oliveira, M.C.N.; Mandarino, J.M.G.; da Silva, J.B.; Ida, E.I.; Carrão-Panizzi, M.C. Changes in the isoflavone profile and in the chemical composition of tempe during processing and refrigeration. *Food Technology* **2011**, *46*, http://doi.org/10.1590/S0100-204X2011001100018.
52. Hutabarat, L.S.; Greenfield, H.; Mulholland, M. Isoflavones and Coumestrol in Soybeans and Soybean Products from Australia and Indonesia. *Journal of Food Composition Analysis* **2001**, *14*, 43–58, http://doi.org/10.1006/jfca.2000.0948.
53. McCue, P.; Horii, A.; Shetty, K. Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by *Rhizopus oligosporus*: Role of carbohydrate-cleaving enzymes. *Journal of Food Biochemistry* **2003**, *27*, 501–514, http://doi.org/10.1111/j.1745-4514.2003.tb00597.x.
54. McCue, P.; Shetty, K. Role of Carbohydrate-Cleaving Enzymes in Phenolic Antioxidant Mobilization from Whole Soybean Fermented with *Rhizopus oligosporus. Food Biotechnology* **2003**, *17*, 27–37, http://doi.org/10.1081/FBT-120019982 .
55. Zhu, L.; Li, W.; Deng, Z.; Li, H.; Zhang, B. The Composition and Antioxidant Activity of Bound Phenolics in Three Legumes, and Their Metabolism and Bioaccessibility of Gastrointestinal Tract. *Foods* **2020**, *9*, http://doi.org/10.3390/foods9121816 .
56. Sriherwanto, C. Recent potential biotechnological applications of the tempe mould *Rhizopus*. A short review. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *759*, 1−11, https://doi.org/10.1088/1755-1315/759/1/012066.
57. Otieno, D.O.; Shah, N.P. Endogenous β-glucosidase and β-galactosidase activities from selected probiotic micro-organisms and their role in isoflavone biotransformation in soymilk. *Journal of Applied Microbiology* **2007**, *103*, 910–917, http://doi.org/10.1111/j.1365-2672.2007.03438.x .
58. Tamam, B.; Syah, D.; Suhartono, M.T.; Kusuma, W.A.; Tachibana, S.; Lioe, H.N. Proteomic study of bioactive peptides from tempe. *Journal of Bioscience and Bioenginnering* **2019**, *128*, 241–248, https://doi.org/10.1016/j.jbiosc.2019.01.019 .
59. Sitanggang, A.B.; Lesmana, M.; Budijanto, S. Membrane-based preparative methods and bioactivities mapping of tempe-based peptides. *Food Chemistry* **2020**, *329*, 1-10, https://doi.org/10.1016/j.foodchem.2020.127193 .
60. Eum, H.L.; Park, Y.; Yi, T.G.; Lee, J.W.; Ha, K.; Choi, I.; *Park,N.I.* Effect of germination environment on the biochemical compounds and anti- inflammatory properties of soybean cultivars. *PLOS One* **2020**, *15*, 1–14, http://doi.org/10.1371/journal.pone.0232159 .
61. Guzmán-Ortiz, F.A.; Martín-Martínez, E.S.; Valverde, M.E.; Rodríguez-Aza, Y.; Berríos, J.D.J.; Mora-Escobedo, R. Profile analysis and correlation across phenolic compounds, isoflavones and antioxidant capacity during germination of soybeans (*Glycine max* L.). *CyTA –Journal of Food* **2017**, *15*, 516–524, https://doi.org/10.1080/19476337.2017.1302995.