Effect of tea extract (*Camellia sinensis*) on shelf life and intrinsic quality of tempeh

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Abstract
Tempeh is Indonesian traditional food made from fermented soybeans. Tempeh has a short shelf life due to its high moisture content and the activity of microorganisms. The shelf life of tempeh can be increased by using tea extract in tempeh processing because tea contains antioxidant and antimicrobial components. This research aims to evaluate the effect of using tea extract in tempeh processing on the shelf life, physicochemical characteristics, and sensory profile of tempeh. During storage, the shelf-life test used the descriptive observation method on the color, flavor, taste, and texture. The use of 0.5% black tea extract in the initial soaking of soybeans can increase the shelf life of tempeh to 4 and 6 days at room and refrigerator temperature, respectively. The result showed that tempeh had significantly (p<0.05) lower ash content (0.81% wb), brightness (65.37), and pH (6.50), while the texture (15.3 Kg force) was significantly higher (p<0.05) than the control (untreated tempeh). The results of sensory evaluation using the hedonic rating test showed that fresh tempeh with black tea extract treatment had significantly lower (p<0.05) color, texture, and overall attributes than the control one. The same evaluation on fried tempeh showed that the black tea extract treatment significantly increased (p<0.05) the texture attribute compared to the control.

1. Introduction
Tempeh is a fermented soybean product that utilizes *Rhizopus* spp. mold. Fermentation causes physical and chemical alterations in soybean, including enhancement of the nutritional and non-nutritional components of tempeh and tempeh flavor. Tempehs are a source of plant-based proteins and isoflavones, both of which are beneficial. The health benefits of tempeh include prevention of diabetes mellitus, osteoporosis, cancer, and reduction of blood cholesterol (1,2). Among the various benefits, tempeh has a short shelf life. Tempeh storage at room temperature lasted for only two days. The limited shelf-life of tempeh is caused by its high moisture content and pH, which is close to neutral, that enabling the growth of microbes (3,4). The damage to tempeh is shown by the color change of the mycelium to brown, as well as the presence of ammonia odor from the continuous fermentation by bacteria (5).
The shelf-life of tempeh can be increased through processing technologies, such as drying, freezing, and utilization of preservative ingredients (6,7). The application of vacuum packaging on seasoned tempeh has increased the shelf-life of tempeh by up to 18 days (8). However, this technology is expensive, making it difficult to apply to tempeh artisans in Indonesia. The submersion of tempeh with tea extract can be utilized to extend the shelf life of tempeh because it has been proven to increase the shelf life of other food products (9–11). The polyphenol components of tea have antioxidant and antimicrobial properties (12,13). Tea antioxidants can also prevent fat and protein oxidation (14,15). As an antimicrobial, tea works both bacteriostatic and fungistatic through enzyme inactivation, membrane cell breakdown, influencing the genetic material function, and chelating the minerals needed for microbial growth (16).

Several factors influence the phenolic compounds in tea. The highest phenolic compounds are found sequentially in white, green, oolong, and black teas (17). The type of tea leaves picked also determined the total phenolic compound. The younger the shoots, the higher its total phenolic is (18). In addition, tea processing determines the total phenolic content. Therefore, this study aimed to determine the tea type, concentration of tea extract, application steps of tea extract utilization in tempeh production, and its effects on physicochemical characteristics, sensory profile, and shelf-life of the treated tempeh.

2. Materials and Methods

2.1. Materials

The main ingredients used in this study were imported non-genetically modified organism (non-GMO) soybeans obtained from KOPTI (Indonesian Tofu and Tempeh Producers Cooperative) Bogor Regency and dry P+2 (green tea and black tea shoots and two young leaves) obtained from the Research Institute for Tea and Cinchona Gambung, Bandung Regency, Indonesia. Non-GMO soybeans were selected because they are widely available and commonly used for tempeh studies, ensuring that the results and findings of our research can be easily replicated and compared with other studies, while also aligning with the common practices for tempeh production. Other ingredients used for tempeh production include yeast and polypropylene plastic packaging. The following chemical substances were used for the analysis: water, non-polar solvent hexane, HCl solution, concentrated H$_2$SO$_4$, HgO, K$_2$SO$_4$, NaOH, Na$_2$S$_2$O$_3$, H$_2$BO$_3$, boiling stoned/maleic anhydride (C$_4$H$_2$O$_3$), methylene red indicator, and methylene blue indicator.

2.2. Determination of Tea Type and Best Tea Extract Application Stage

The type of tea and application stage of the tea extract were determined by producing six types of tempeh from the combination of two kinds of tea. The types of tea used were black and green. Tea extract was applied in the first submersion stage for 90 min, soybean boiling stage for 30 min, and second submersion stage (12 h). The concentration of extracted tea was 1% (1 g tea per 100 mL boiling water). The ratio of soybean to tap water used during the tempeh processing was 1:2 (b/v). The tea type and application of the best tea extraction method were determined by analyzing the color parameter, texture, aroma, and tempeh flavor for 4 days at room temperature. The color and texture parameters were analyzed objectively using a spectrophotometer and texture analyzer, while aroma and taste parameters were analyzed using a descriptive method.
2.3. Determination of The Best Tea Extract Concentration

The best tea extract concentration was determined after determining the tea type and application of the best tea extract. Four types of tempeh were produced based on the tea extract concentration (0-1%): tempeh A (0%), tempeh B (0.5%), tempeh C (0.75%), and tempeh D (1%). The optimal tea extract concentration was determined by analyzing the color, aroma, and taste of tempeh during 4 days of storage at room temperature. The color parameter was analyzed objectively using chromomter, while the aroma and taste parameters were determined using the descriptive method, which involved 70 untrained panelists to evaluate and describe the sensory attributes of the samples.

2.4. Tea Extraction

The tea extraction (dry soybean base 500 g) was initiated by inserting 6 g of dry tea into 1.2 L of boiling water and steeping for an hour, allowing for the optimal dissolution of tea compounds. The solution extract was then strained to obtain the tea extract for soybean submersion and boiling during tempeh production.

2.5. Tempeh Production with Tea Extract

The tempeh production process is referred to as Rumah Tempe Indonesia (RTI, a hygienic tempeh house production model located in Cilendek, Bogor, West Java). Tempeh production using tea extracts was initiated by sorting and cleaning. The soybean was then washed, and the first soybean soaking stage for 90 min was performed using the tea extract. The tea extract to dry soybean ratio was 1:2 (b/v). The soybean was then strained from its soaking water and boiled with soaking water overnight (12 h). The soybean was then separated from its outer skin and spliced into two parts using a dehuller machine, washed, and poured with hot water at 100°C. The soybeans were then strained and air-dried. Dried soybean was inoculated with yeast (2 g of yeast/1 kg soybean) and then packed into perforated polypropylene plastic with a distance between holes of 2 × 2 cm. The final step was to store the mixture of soybean and yeast in a fermentation chamber (at 30°C, RH 80%) for 40 h until it became tempered.

2.6. Proximate Analysis

Proximate analysis was conducted for both tempeh types: tempeh with tea extract addition and tempeh without tea extract (control). The proximate analysis was based on AOAC (19), which consists of moisture content (oven method), ash content (dry ash method), crude protein content (Kjeldahl method), fat content (Soxhlet method), and carbohydrate content calculated by difference.

2.7. Color and pH Analysis

Color and pH analyses were performed for both tempeh types. Color testing was performed using a chromometer CR 300 Minolta instrument. The color measurement data were the L values. The pH was measured using a pH meter. A sample of 10 g was mashed, and 10 mL of distilled water was added to measure its pH (20).
2.8. Texture Analysis
Texture analysis was performed for both the tempeh types. Texture analysis was performed using texture analyzer TA-XT2. A sample size of 2.5 x 2.5 cm was analysed using a knife probe with a probe speed of 1.5 mm/s, 30 mm distance, and trigger force of 205 g (21).

2.9. Sensory Analysis
The sensory analysis evaluation was performed using the hedonic rating method with 70 untrained panelists. The tested samples were then fresh and fried. The tested sensory attributes for fresh tempeh were color, aroma, texture, and overall, whereas the sensory attributes for fried tempeh were color, aroma, taste, texture, and overall. The evaluation was performed by assigning a score based on a scale of 1–7.

2.10. Determination of Tempeh Shelf-Life
Shelf-life analysis was performed using a descriptive method to observe the color, aroma, texture, and taste during storage. The shelf-life of tempeh was determined by observing the condition of tempeh during storage and comparing it with the characteristics of good-quality tempeh based on SNI 3144:2015. Good quality tempeh has the following characteristics: [1] it has an evenly distributed white color on the whole surface and no greyish black mold spores found; [2] it has a distinctive tempeh odor and has not yet formed an ammonia odor; and [3] it does not crumble easily when sliced. Tempehs that do not match these characteristics can experience spoilage.

2.11. Data Analysis
Data were analyzed using Microsoft Excel and SPSS 22. Data are presented as the average value ± standard deviation. The tea types, tea extract application stage, and tea extract concentration data were analyzed using Univariate ANOVA and post-hoc Duncan with a 95% confidence interval. Proximate analysis data, physical analysis (color, texture, and pH), and sensory analysis were analyzed using an independent sample t-test with a 95% confidence interval.

3. Results and Discussion
3.1. Determination of Types of Tea and Best Tea Extract Application Stage
The tempeh produced consisted of a tempeh with black tea extract added to the first soaking stage (BS-1), green tea extract-added tempeh in the first soaking stage (GS-1), black tea extract-added tempeh during the boiling stage (BB), green tea extract-added tempeh during the boiling stage (GB), black tea extract-added tempeh in the second soaking stage (BS-2), and green tea extract-added tempeh in the second soaking stage (GS-2). The color analysis results (lightness) are listed in Table 1, and the physical appearance of the tempeh is shown in Figure 1. The tempeh with tea extract treatment offered the best lightness during the second day of storage, which was the BS-1 and GS-1 tempeh. Statistical analysis showed that on the second day of storage, the BS-1 and GS-1 tempehs had a similar lightness (p>0.05) to the control tempeh (Table 1).
Table 1. Lightness (L-value) of tempeh during storage at room temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS-1</td>
<td>64.45±0.90</td>
<td>67.89±1.30</td>
<td>64.56±2.23</td>
<td>60.52±1.90</td>
<td>62.27±2.96</td>
</tr>
<tr>
<td>GS-1</td>
<td>66.17±1.56</td>
<td>62.66±1.10</td>
<td>64.11±3.10</td>
<td>54.58±0.81</td>
<td>53.20±1.00</td>
</tr>
<tr>
<td>BB</td>
<td>62.13±1.77</td>
<td>61.82±2.04</td>
<td>56.66±0.42</td>
<td>58.50±4.96</td>
<td>58.13±0.14</td>
</tr>
<tr>
<td>GB</td>
<td>65.58±61.79</td>
<td>62.66±0.48</td>
<td>54.96±0.20</td>
<td>54.82±0.84</td>
<td>45.84±5.84</td>
</tr>
<tr>
<td>BS-2</td>
<td>59.08±1.92</td>
<td>64.41±0.84</td>
<td>63.33±0.44</td>
<td>56.51±1.44</td>
<td>47.43±1.44</td>
</tr>
<tr>
<td>GS-2</td>
<td>56.95±1.00</td>
<td>62.73±1.03</td>
<td>55.72±1.40</td>
<td>55.77±1.25</td>
<td>51.82±1.01</td>
</tr>
</tbody>
</table>

BS-1: tempeh treated with black tea extract on the first soaking stage, GS-1: tempeh with green tea extract treatment on the first soaking stage, BB: tempeh with black tea extract treatment during boiling stage, GB: tempeh with green tea extract treatment during boiling stage, BS-2: tempeh with black extract treatment on the second soaking stage, GS-2: tempeh with green tea extract treatment on the second soaking stage) at room temperature storage. Mean values followed by the same letters are not significantly different (p>0.05).
Overall, the lightness value of each tempeh type deteriorated during storage. Based on the statistical analysis results (Table 1), tempeh with tea extract treatment with the best lightness during storage was the BS-1 and GS-1 tempeh on day 2 of storage. The results showed that green tea and black tea extracts retained the tempeh color during storage. Kim et al. (22) reported that the soaking process with tea extract could increase the lightness value and yellow color intensity of soybeans. Tea’s antioxidant compounds are also capable of preventing lipid oxidation, which results in color changes (23). Wang et al. (24) also reported that fish treatment with tea polyphenols could inhibit the decrease in L* owing to a reduction in the oxidation process. The oxidation of proteins and lipids can change light reflectance and produce substances that affect color (25).

Statistical analysis (Table 2) showed an interaction between storage time and the type of tempeh that affected the tempeh texture (hardness). The GS-2 tempeh did not experience significant texture change (p>0.05) after four days of storage; thus, based on the texture parameter, the GS-2 tempeh was tempered with the best treatment. This result can be attributed to the interaction between soy protein and tea polyphenol, which enhances texture characteristics (26). Typically, a tempeh experiences a change in texture to a softer and watery texture during storage. This was caused by further fermentation by bacteria. Further fermentation can damage the soybean matrix and result in a tender texture (8). During further fermentation, hydrolysis of carbohydrates, proteins, and fats occurs, which results in a tender tempeh texture and pungent odor (27). Tea polyphenol treatment in fish could also effectively retain the hardness of refrigerated bass flesh during storage due to the inhibition of microorganism growth, resulting in lower bacterial load and lighter degradation in fish meat (24).

In addition to objective analysis, descriptive analysis of the aroma and taste of the six types of tempehs was performed. The aroma parameter analysis results showed that tempeh treated with black tea extract and green tea extract did not generate ammonia until the fourth day of storage. Fried BS-1, GS-1, BB, and GB tempehs had a common taste, but after the third day of storage, the taste became slightly bitter. BS-1 had a taste almost similar to that of the control tempeh. Based on the objective and descriptive analysis results, BS-1 was
found to be the best treatment. The BS-1 tempeh texture increased during storage, but remained acceptable.

Table 2. Hardness value (g force) of tempeh during storage at room temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days 0</th>
<th>Days 2</th>
<th>Days 3</th>
<th>Days 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS-1</td>
<td>17310±</td>
<td>25645±</td>
<td>22650±</td>
<td>31735±</td>
</tr>
<tr>
<td>GS-1</td>
<td>1159.66 df</td>
<td>1223.29 i</td>
<td>2687.01 h</td>
<td>1760.70 j</td>
</tr>
<tr>
<td>BB</td>
<td>15730±</td>
<td>19105±</td>
<td>22470±</td>
<td>27515±</td>
</tr>
<tr>
<td>GB</td>
<td>410.12 de</td>
<td>1011.16 fg</td>
<td>707.11 h</td>
<td>756.60 i</td>
</tr>
<tr>
<td>BS-2</td>
<td>18195±</td>
<td>12535±</td>
<td>21810±</td>
<td>17085±</td>
</tr>
<tr>
<td>GS-2</td>
<td>1039.45 efg</td>
<td>586.90 bc</td>
<td>933.38 h</td>
<td>926.31 def</td>
</tr>
</tbody>
</table>

BS-1: tempeh treated with black tea extract on the first soaking stage, GS-1: tempeh with green tea extract treatment on the first soaking stage, BB: tempeh with black tea extract treatment during boiling stage, GB: tempeh with green tea extract treatment during boiling stage, BS-2: tempeh with black extract treatment on the second soaking stage, GS-2: tempeh with green tea extract treatment on the second soaking stage at room temperature storage. Mean values followed by the same letters are not significantly different (p>0.05).

3.2. Determination of The Best Tea Extract Concentration

The best tea extract concentration was determined by objectively analyzing the color using a chromameter and descriptively observing the color, aroma, and taste. The best lightness of the tea-treated tempeh extract was higher or similar to that of the control tempeh. The black tea extracts used in the first boiling stage varied from 0 to 1%, specifically 0% (tempeh A), 0.5% (tempeh B), 0.75% (tempeh C), and 1% (tempeh D). All four tempehs were stored for two days at room temperature, and the color was analyzed with a chromameter. The analysis results are listed in Table 3, and the physical appearances of tempehs A, B, C, and D are displayed in Figure 2. The statistical analysis results showed an interaction between storage and type of tempeh on the lightness (L value) of tempeh. Tempeh C at day 0 and day 1 of storage had similar lightness values (p>0.05) to tempeh A (control) on day 0. Meanwhile, on day 2 of storage, tempeh B had a similar lightness value to that of tempeh A.

Table 3. Lightness (L-value) of tempeh on the second day of storage at room temperature.

<table>
<thead>
<tr>
<th>Tempeh</th>
<th>Days 0</th>
<th>Days 1</th>
<th>Days 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tempeh A</td>
<td>71.95±0.73 f</td>
<td>60.39±0.23 abc</td>
<td>57.65±0.91 a</td>
</tr>
<tr>
<td>Tempeh B</td>
<td>63.76±2.18 bcd</td>
<td>60.86±1.05 abc</td>
<td>58.88±2.43 ab</td>
</tr>
<tr>
<td>Tempeh C</td>
<td>68.80±5.26 def</td>
<td>68.45±1.06 def</td>
<td>63.96±2.20 bcde</td>
</tr>
<tr>
<td>Tempeh D</td>
<td>64.45±0.90 cde</td>
<td>64.70±3.12 cde</td>
<td>69.13±0.11 ef</td>
</tr>
</tbody>
</table>

Tempeh A = 0% black tea extract, Tempeh B = 0.5% black tea extract, Tempeh C = 0.75% black tea extract, Tempeh D = 1% black tea extract. Mean values followed by the same letters are not significantly different (p>0.05).
Tempeh A: 0% black tea extract, B: 0.5% black tea extract, C: 0.75% black tea extract, D: 1% black tea extract at room temperature storage.

The aroma parameter analysis results showed that ammonia odor was not detected in tempehs B, C, and D until day 4 of storage. Taste parameter analysis results showed that fried tempehs A, B, C, and D had a similar aroma and taste to tempeh. However, an ammonia odor on fried tempeh A was detected on day 1 of storage. Based on objective and descriptive analyses, it was determined that tempeh with 0.5% black tea (tempeh B) was the best tempeh. Too much tea extract can result in decreased lightness and increased off-flavors in tempehs. This treatment was used in the subsequent analysis stage.

3.3. Chemical Composition and Physical Characteristics

Proximate analysis was performed to determine the chemical composition of the samples, including moisture, ash, protein, fat, and carbohydrate (by difference) contents. Tempeh B was selected for further analysis based on the results of objective and descriptive analyses, indicating that it exhibited similar lightness to tempeh A as a control, and thus could be used for subsequent comparison. The statistical analysis (Table 4) showed that the moisture, protein, fat, and carbohydrate contents between tempehs A and B were not significantly different (p>0.05). However, in terms of ash content, Tempeh B was significantly lower (p<0.05) than Tempeh A. This was due to the use of the black tea extract as the first soaking solution for 90 min. The phenolic content of black tea binds to tempeh minerals and some free minerals, which are drained during the cleaning process. As a result, the ash content of the tempeh is reduced. Kim et al. (22) reported a similar result, which indicated that some minerals were water-soluble when the soybean was cleaned along with the tea extract. The minerals found in tempehs are iron, calcium, phosphor, zinc, and magnesium (28).
Table 4. The chemical and physical characteristics of tempeh A and tempeh B.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tempeh A</th>
<th>Tempeh B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>64.04±0.08 a</td>
<td>64.19±0.28 a</td>
</tr>
<tr>
<td>Ash</td>
<td>1.04±0.01 b</td>
<td>0.81±0.01 a</td>
</tr>
<tr>
<td>Protein</td>
<td>19.49±0.47 a</td>
<td>18.96±0.51 a</td>
</tr>
<tr>
<td>Fat</td>
<td>8.08±0.08 a</td>
<td>8.66±0.14 a</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>7.36±0.47 a</td>
<td>7.40±0.07 a</td>
</tr>
<tr>
<td>Color L</td>
<td>71.81±0.57 b</td>
<td>65.37±3.18 a</td>
</tr>
<tr>
<td>Color a</td>
<td>3.17±0.45 a</td>
<td>3.69±0.98 a</td>
</tr>
<tr>
<td>Color b</td>
<td>16.59±1.14 a</td>
<td>19.14±2.25 a</td>
</tr>
<tr>
<td>Texture (g force)</td>
<td>10819.00±914.47 a</td>
<td>15290.00±970.62 b</td>
</tr>
<tr>
<td>pH</td>
<td>7.77±0.06 b</td>
<td>6.50±0.00 a</td>
</tr>
</tbody>
</table>

Tempeh A: 0% black tea extract, B: 0.5% black tea extract. Mean values followed by the same letters are not significantly different (p>0.05).

The analyzed physical characteristics consisted of color, texture, and pH. Based on the L value parameter, tempeh B had a darker color than tempeh A (p<0.05). This was attributed to the effect of catechin from black tea. During processing, catechin undergoes oxidation and turns into theaflavin and thearubigin, which provide color to the tea brew (29,30). Mao et al. (31) reported a similar result, stating that the interaction between a protein with theaflavin and thearubigin could change the color following the pigment in black tea. Theaflavin contributes to the formation of a yellow-brownish color, whereas thearubigin contributes to the formation of a red-brownish color (32).

The hardness value indicates the amount of force required to press a sample. The hardness of tempeh B was much higher than that of tempeh A (p<0.05). This was due to the interaction between the phenolic compound and the tempeh protein. Ou et al. (33) stated that the interaction between phenolic compounds, wheat protein, and tapioca flour influences the texture of bread products. However, the underlying mechanism remains unknown.

The pH of tempeh B was significantly lower (p<0.05) than that of tempeh A. This was because of the use of black tea extract as soybean soaking water in tempeh B. The utilization of tea could decrease the pH of tempeh due to its acidic characteristics, with a pH ranging from 4.7 to 5.2 (34). Black tea is sour because it undergoes perfect enzymatic oxidation during processing, which results in the conversion of oxidized catechin into theaflavin and thearubigin, which determine the acidity of black tea (35). The texture difference between tempehs A and B was caused by the interaction between phenolic components from the tea in tempeh B, which was suspected to affect the tempeh texture. Several studies have shown that the interaction between soy protein isolates and polyphenols can increase the texture characteristics of food products (26,36).

3.4. Sensory Analysis

Sensory differences in the color, texture, and overall quality of tempeh are linked to consumer preference. Consumers typically hold specific expectations regarding the color and texture of a common tempeh; therefore, any notable deviation from these expectations can influence their perception. These differences may arise from variations in tempeh production.
methods. Consequently, we conducted a sensory analysis to determine consumer preferences for tempeh produced using the tea extract-soaking process. Statistical analysis of fresh tempeh (Table 5) showed that the assessment of color, texture, and overall tempeh B was significantly lower (p<0.05) than that of tempeh A. This result implied that the color, texture, and overall tempeh A were preferred over tempeh B. Statistical analysis of fried tempeh showed that the sensory evaluation result of fried tempeh B was higher (p<0.05) than that of tempeh A, which showed that the texture of fried tempeh B was more preferred than that of fried tempeh A.

Table 5. The sensory test result of fresh tempeh and fried tempeh from tempeh A and tempeh B.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Tempeh A</th>
<th>Tempeh B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh tempeh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>6.13±0.78 b</td>
<td>5.50±1.21 a</td>
</tr>
<tr>
<td>Aroma</td>
<td>5.43±1.19 a</td>
<td>5.44±1.04 a</td>
</tr>
<tr>
<td>Texture</td>
<td>5.90±0.68 b</td>
<td>5.43±1.15 a</td>
</tr>
<tr>
<td>Overall</td>
<td>5.97±0.66 b</td>
<td>5.53±0.85 a</td>
</tr>
<tr>
<td>Fried tempeh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>5.77±1.05 a</td>
<td>5.49±0.86 a</td>
</tr>
<tr>
<td>Aroma</td>
<td>5.84±0.97 a</td>
<td>5.76±1.04 a</td>
</tr>
<tr>
<td>Texture</td>
<td>5.07±1.28 a</td>
<td>5.56±1.02 b</td>
</tr>
<tr>
<td>Taste</td>
<td>5.39±1.29 a</td>
<td>5.47±1.07 a</td>
</tr>
<tr>
<td>Overall</td>
<td>5.56±0.97 a</td>
<td>5.64±0.80 a</td>
</tr>
</tbody>
</table>

Tempeh A: 0% black tea extract, B: 0.5% black tea extract. Mean values followed by the same letters are not significantly different (p>0.05).

3.5. Shelf-Life of Extract Tea-Treated Tempeh

The shelf-life analysis results showed that tempehs with black tea extract stored at room temperature had acceptable sensory attributes (color, aroma, taste, and texture) up to day-4 of storage. On day 5 of storage, the black tea-treated tempeh on day-5 started to produce an ammonia odor mixed with the tea scent, the appearance of black spots on the soybean, the formation of a yellow-brownish color on the tempeh mycelia, and a softer texture. Tempeh treated with black tea extract stored in the refrigerator (at 5°C) had acceptable sensory attributes up to day-6. The tempeh with tea extract on day-7 of storage had a sour aroma, its mycelium started to turn yellow, and the texture became tender.

The application of tea extract in tempeh processing, which can extend shelf life, is suspected to be caused by several factors. The first factor is the phenolic component of tea, which inhibits further fermentation during tempeh processing (37). Additional fermentation results in an ammonia odor and a tender tempeh texture. The second factor is the antioxidant activity of the phenolic components of the tea. The antioxidant activity of tea works in several ways, such as binding free radicals, inhibiting enzyme activity, or working synergistically with other antioxidants (38). The third factor is the antimicrobial activity of the phenolic compounds. Microbial activity extends the shelf life of tempehs (11). Similar results were also found in a study by Pakfetrat et al. (39), which explained that applying tea extract could reduce the initial microbial load on legumes and extend their shelf life. Commonly, antimicrobial compounds in food products act as bacteriostatic agents that prevent or inhibit bacterial growth (40).

Black tea extract has been shown to extend the shelf life of tempeh by up to 4 days, and it has potential economic implications for both tempeh producers and retailers. Tempeh
producers will reduce the costs associated with spoilage and the number of products that go to waste. Furthermore, they can charge a premium for tempehs with a longer shelf life and sell to a wider range of customers. Retailers can also benefit from selling tempehs with a longer shelf life, as they can minimize losses associated with unsold or spoiled tempehs. Overall, businesses and consumers can benefit from tempehs with an extended shelf life. Businesses can save money on disposal costs and increase revenue by selling more products, whereas consumers can save money on their grocery spending by purchasing fewer products and experiencing diminished product waste.

4. Conclusions
Soybean soaking with 0.5% black tea extract increased the shelf-life of tempeh by up to 4 days at room temperature and 6 days in the refrigerator. Tempeh with 0.5% black tea extract was identified as the best treatment because of its optimum lightness and flavor compared to 0.75%, 1%, and the control tempeh. Tempehs with 0.5% black tea extract had lower lightness and pH values and higher hardness values than the control. Tempeh treated with 0.5% black tea extract had moisture, protein, ash, and carbohydrate content, which were not different from the control. Tempeh with 0.5% black tea treatment had color, texture, and overall attributes that were less preferred, but the texture of fried tempeh with 0.5% black tea extract treatment was preferred over the control tempeh. Black tea extract is a promising natural additive for tempeh not only because of its remarkable shelf-life extension but also because of its inherent antioxidant properties, which have been associated with numerous health benefits. However, further research is needed to confirm the extent of the antioxidant increase and to determine the optimal concentration of tea extract for use in the tempeh-making process.

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Author Contributions
M.A. and T.W. conceived and designed the experiments; P.H. performed the experiments and analyzed the data; A.P.G.P interpreted the data and wrote the paper; M.A. and A.E.F reviewed and provided critical feedback.

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The sensory evaluation in this study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee on Research Involving Human Beings of IPB University.
Data Availability Statement
Invalid.

Conflicts of Interest
No conflict of interest.

References


