EVALUATION OF SOYBEAN VARIETIES ON PRODUCTION AND QUALITY OF TEMPE

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ABSTRACT. Tempe have been an important part of Indonesian diet for hundred years and eaten by all sector of the population; in villages as well as cities. Nutritionally, tempe represent food rich in proteins and also contains many other important nutrients. The present research aimed to evaluate varieties of soybean on the quality of tempe. Four soybean varieties namely US IP soybean; US soybean; Argentina soybean; and Local soybean were evaluated in this study. The research also included IP soybeans that had been stored for 1 year, thus the total soybeans evaluated were 5 soybean samples. Tempe was evaluated for yield, moisture, protein, fat, total solid contents, water absorption and retention, and sensory characteristics. The results show that the soybean varieties affected yield protein and fat content, and sensory characteristics of tempe. IP soybean absorbed more water than the other varieties after 48 h soaking during tempe preparation. Storage of IP soybean for 1 year did not greatly affected yield of tempe, and protein content, however it affected the sensory characteristics. Tempe produced from fresh IP-soybean was the most preferred sensory quality. On nutritional aspect, however, tempe of local soybean had the highest protein content than that of tempe of other varieties of soybean.

Keywords: soybean varieties, tempe, protein content, fat content, sensory characteristics

INTRODUCTION

Tempe is Indonesian traditional fermented food made of soybean. Tempe has been an important part of the Indonesian diet for hundred of years and eaten by all sector of the population; in villages as well as cities. Nutritionally, tempe represents a food rich in proteins and also contains many other important nutrients such as vitamin B12 and minerals. Its excellent protein quality has made tempe becoming in-expensive substitutes to meat.

The craft of tempe making is passed on through generations of tempe making families. Basically tempe is made from cooked soybean that have been
inoculated by certain mold spore or starter culture called *laru* or *ragi*. In some area, leaf of *Hibiscus tilacius* that has been overgrown by spores, called *usar* is used as starter culture. Many microorganisms including molds and bacteria present in laru, however the most important species for making tempeh is *Rhizopus oligosporus* (Pawiroharsono, 1996). Other *Rhizopus* such as *R. stolonifer*, *R. arrhizus* other mold such as *Mucor* are sometime present in tempe starter, and may also contribute in fermentation of tempe.

Prior to coking of soybean and inoculation of cooked soybean, the soybean is soaked overnight. During soaking, acid producers organism such as lactic acid bacteria grow to acidify the bean. The inoculated cooked soybean are packed into banana leaves or perforated plastic bag and then left to ferment. During this time, the mold grows through to knit together the beans and turn them into tempe. Tempe is usually harvested after 36-48 h fermentation. When the fermentation is prolonged, the mold will continue to grow, produce the spores and make tempe turn into black. Subsequent growth of tempe will also continuously break down the nutrition and food component. This will result in off odor due to the subsequent breakdown of protein into ammonia, and texture of tempe becoming soft.

Beside soybeans, others beans have also been used to produce tempe in limited area, especially in Java. Koro benguk (*Mucina pruriens* L.D.C. var utilis) is the second choice of beans for tempe after soybeans. This kind of tempe is popular especially in Mid Java. Winged beans and mungbeans are also used for tempe. Due to limitation in raw material, currently only soybean’ tempe that widely made and consumed.

The quality of tempe depend on the starter culture quality and the bean used as raw material. Used of different bean will need some modification in processing. The objective of this activity is to evaluate varieties of soybean on the quality of tempe, and to evaluate mirobial changing during soaking and fermentation.

**METHODOLOGY**

**Raw Material**

The raw material for producing tempe four varieties of soybean, i.e. US IP soybean; US No. 1 soybean; Argentina soybean; and Local soybean. For making
tempe also included US IP soybean that had been stored for 1 year. All variety of soybean are supplied from American Soybean Association (ASA), International Marketing, and analysed for protein, fat and water content.

**Production of Tempe**

The way of making tempe was started by washed one kilograms of soybean, boiled for 20 minutes, cooled and allow for soaking for 48 h. After soaking, the soybeans were peeled their husks, washed and steamed for 20 minutes and cooled. Afterwards, the steamed soybean was inoculated with tempe starter culture (*laru*) as much as 5 gram/kg of soybean. The inoculated soybeans were packed as much as 350 gram in perforated plastic bag and fermented for 48 hours until they turn into tempe. Analyses were conducted on yield of tempe, protein, fat, moisture and total solid content, sensory characteristic, water absorption and weight loss during storage of tempe.

Separate experiment using US soybean was conducted to evaluate the involvement of bacteria during fermentation of tempeh. Analyses were carried out for microbial changing i.e. total plate count and total lactic acid bacteria in soaking water and soybean after 1 and 2 days soaking, and during fermentation process. During incubation, total mold was also analysed.

**Methods for Analysis**

The methods of analysis for quality of tempe cover two parameters, i.e. the chemistry and sensory quality.

*Yield*

Total of product weight was measured by analytical scale thus the yield was calculated by following formula:

\[
\text{Yield} = \frac{\text{Product weight (gram)}}{\text{Soybean weight (gram)}} \times 100\%
\]

*Moisture content*

Moisture content was analysed using the method of AOAC (MD Method 950.46). Aluminium cup was dried in oven at temperature 102°C for 20 minutes, cooled in desiccator for ten minutes. Afterwards, cup was weighed using
analytical scale (a gram). As much as 2 gram of sample was weighed (x gram), placed in the dried cup and dried in oven at temperature 102°C for 16 hours, cooled in a dessicator and weighed until the weight is constant (y gram).

\[ \text{Moisture content (\%)} = \frac{x - (y - a) \times 100}{x} \]

**Protein content (AOAC, 1984)**

Sample was weighed as much as 0.1-0.15 gram, placed in the destruction flask, added 1.910.1 g K₂SO₄, 40110 mg HgO and 2±0.1 ml H₂SO₄. If it is more than 15 mg, then 0.1 ml H₂SO₄ was added to every 10 mg organic substances. And then, added some boiled stones. Sample was destructed for 1-1.5 hours or until the liquid turn clear, and cooled. After it was cooled, slowly added a number of water and cooled back. The volume was moved into distilled flask, rinsed 5-6 times with 1-2 ml water. Prepared the Erlenmeyer flask 125 ml containing 5 ml H₂BO₃ solution and 2-4 drips of indicator (mixture of 2 part of red methyl 0.2% in alcohol and 1 part of methylene blue 0.2% in alcohol) and put it under the condenser. The tip of condenser must be immersed in H₂BO₃ solution. And then, added 8-10 ml NaOH-Na₂SO₃ solution, distilled until it was accommodated about 15 ml. The condenser tube was rinsed with aquades and it was accommodated in the same erlenmeyer flask. The volume in the erlenmeyer flask was diluted until about 50 ml, and then conducted by a titration with HCl 0.02N until the color change into grey. After determined the sample, it was also done for the blanko.

\[ \% \text{ Protein (db)} = \frac{(\text{ml HCl} - \text{ml blanko}) \times N \text{ HCl} \times 14.007 \times 100} {\text{mg sample (db)}} \times 6.25 \]

**Fat content (AOAC, 1984)**

Soxhlet flask was dried in oven, cooled in desiccator and measured. As much as 2 gram of sample powder was put into filter paper with appropriate mesh and covered by cotton. Filter paper which contained of sample was added into Soxhlet-extraction machine, built the condenser on the top and the Soxhlet flask on the bottom. And then, hexane was added into the Soxhlet flask sufficiently, refluxed for 5 hours minimum until the solvent return to clear. Soxhlet flask which contained fat was heated in oven at temperature 105°C. After it was dried until its constant weight in desiccator, the Soxhlet flask was measured.

\[ \% \text{ Fat content (db)} = \frac{\text{Fat weight (gram)}}{\text{Sample weight (db)} (gram)} \times 100 \]
I-Evaluation of Soybean Varieties on Production and Quality of Tempe

**Total solid**

Total solid was calculated by using this following formula:

\[
\text{Total solid} = \frac{\text{Total product weight} - \text{Water weight in the product}}{\text{Total weight product}} \times 100\%
\]

**Water absorption**

Determination of water absorption value was conducted by calculating the difference between soybean weight after soaking process for 48 hours and total first soybean weight.

**Weight loss**

Determination of weight loss value was conducted by cut the product into two parts and let them at room temperature 27°C and relative humidity (RH) 70% for 9 hours. Every 3 hours, the product was weighed. The difference between first product weight and its weight after 9 hours was determined as a product weight loss.

**Sensory analysis**

Sensory analysis for tempe was conducted by Intensity Rating Test which cover parameters, i.e. color, beany flavor, nutty flavor, acid aroma, bitterness, sour taste and after taste. Sample was given randomly to 30 - 45 non-trained panellists. The data was processed by *Balance Inblock Design*.

**Microbial analyses**

Total plate was analysed using PCA (BAM, 2001a), lactic acid bacteria was counted in MRS agar, while total mold was counted in PDA supplemented with chloramphenicol (BAM, 2001b).

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RESULT AND DISCUSSION

**Analyses of Soybean**

The soybeans which were used for this study was supplied from American Soybean Association (ASA), International Marketing. There were four varieties of soybean used for making tempe, tofu and soymilk, i.e. US IP soybean, US No. 1
soybean, Argentina Soybean and Local Soybean. In this research also used soybean that had been stored for a year. Figure 1.1 clearly demonstrates that local soybean had the smallest shape than others.

![Figure 1.1. Different varieties of soybean](image)

Table 1.1 shows that the moisture content of those five varieties of soybean is differ. Local soybean had the highest moisture, followed by Argentina soybean, US IP soybean 1 year old. US No. 1 soybean, and Fresh US IP soybean. According to that, it was obvious that US IP soybean undergo moisture content increment after one year of storage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Fresh US IP</th>
<th>US IP 1 year old</th>
<th>US No.1</th>
<th>Argentina</th>
<th>Local</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>%</td>
<td>8.67</td>
<td>9.07</td>
<td>8.68</td>
<td>9.71</td>
<td>9.95</td>
</tr>
<tr>
<td>Protein (db)</td>
<td>%</td>
<td>5.35</td>
<td>5.30</td>
<td>5.15</td>
<td>5.36</td>
<td>5.16</td>
</tr>
<tr>
<td>Fat (db)</td>
<td>%</td>
<td>20.58</td>
<td>19.50</td>
<td>25.11</td>
<td>19.60</td>
<td>15.91</td>
</tr>
</tbody>
</table>

Mean of protein content value on five varieties of soybean was about 5%. Argentina soybean had the highest protein content (Table 1.1) followed by Fresh US IP soybean, US IP soybean 1 year old (5.30%), local soybean and the lowest was US No. 1 soybean. Meanwhile, the US No. 1 soybean had the highest fat content, followed by Fresh US IP soybean, Argentina soybean, US IP soybean 1 year old, and local soybean had the lowest fat content (Table 1.1). Storage the US IP soybean did not affect the protein content.
Tempe

Yield of tempe

The research results (Figure 1.2) show that the highest yield of tempe was made from Fresh US IP soybean, that was not different significantly with US IP soybean 1 year old, followed by US No. 1 soybean, Argentina soybean, and the lowest one was local soybean. Storage of the bean for 1 year did not affect the yield of tempe.

Figure 1.2. Yield of tempe from different varieties of soybean

Visual observation during fermentation showed that the molds grew faster in local soybean as reflected in its appearance after 24 h fermentation where the cottony mycelium has covered the bean. This probably related to the smaller bean of local soybean as compared to the other varieties. The appearance of tempe made from different variety of soybean is depicted in Figure 1.3.

Figure 1.3. Tempe from different varieties of soybean
Moisture Content of Tempe

The research results (Figure 1.4) show that tempe made from Argentina soybean had the highest moisture content, followed US IP soybean 1 year old, US No. 1 soybean, Fresh US IP soybean, and the lowest one was local soybean, however the range of the moisture content of tempe is narrow (59.11-62.07%). This results show that there was no correlation between the moisture content of tempe and the yield.

![Figure 1.4. Moisture content of tempe from different varieties of soybean](image)

Protein Content of Tempe

Figure 1.5 shows that tempe made from local soybean had the highest protein content, followed by US IP soybean 1 year old, Fresh US IP soybean, US No. 1 soybean and Argentina soybean. Storage of the soybean for 1 did not affect the protein content of tempe.
Those data were calculated based on dry basis. Local soybean that provided lowest yield of tempe, has the highest protein content of tempe. In general, the protein content of tempe was higher than that of protein content of soybean, however, the protein content of tempe did not correlate with the protein content of soybean (Figure 1.6). The mycelium that is basically mycoprotein, may contribute to the increase of protein content. The proteolytic
activity of the proliferating mycelium also brings about nutritionally significant consequences such as increase in digestibility of tempe as compared to soybean (Rahayu, 2004).

**Fat content of tempe**

The result of fat content of tempe from five varieties of soybean was shown on Figure 1.7. The highest fat content of tempe was made from Argentina soybean, followed by Fresh US IP soybean, US No. 1 soybean, US IP soybean 1 year old, and the lowest one was local soybean. Those data were calculated based on dry basis. Storage of soybean for 1 year decreased the fat content in tempe significantly. During fermentation, fatty acids are deliberated, resulting in hydrolysis of over 30% neutral lipid (Wagenknecht, et al., 1961). Comparing the fat content of soybean (Table 1.1) and the fat content of tempe shows that the fat content of tempe was lower than the fat of the soybeans, that decreased almost half of that in the soybeans. This reflects that the mold growth during fermentation has quite active lipolytic activity.

![Figure 1.7. Fat content of tempe from different varieties of soybean](image)

**Total solid of tempe**

Figure 8 demonstrated that tempe made of local soybean had the highest total solid value which was 40.89%, followed by Fresh US IP soybean (39.93%), US
No. 1 soybean (39.18%), US IP soybean 1 year old (38.42%) and the lowest one was Argentina soybean (37.93%).

During fermentation, biochemical changes occur as results of enzymes activity. Beside, cahning in protein and fat, molds also digest carbohydrates. The principal changes in carbohydrates are rapid removal of hexoses and slow hydrolysis of stachyose (Rahayu, 2004). This biochemical changes affects the total solid of tempe.

![Figure 1.8. Total solid of tempe from different varieties of soybean](image)

**Water absorption of tempe**

The US IP soybean 1 year old has the highest water absorption, but no significant different with Fresh US IP (Figure 1.8), followed by local, US No. 1 and Argentina soybean. The result of water absorption of tempe was shown on Figure 9. The US IP soybean also gave the highest yield (Figure 1.2).
Weight loss of tempe

The research result that tempe made by local soybean had the biggest weight loss which was 10.66%, followed by Fresh US IP soybean (9.86%), Argentina soybean (9.49%), US No. 1 soybean (9.32%), and US IP soybean 1 year old (8.70%). The result of water absorption of tempe was shown on Figure 1.10.
Sensory quality of tempe

Figure 1.11 shows that sensory characteristic of tempe made from five varieties of soybean had a different pattern. Tempe made from Fresh US IP soybean had the best characteristic in comparison to other soybeans because it had the lowest characteristic of beany flavor, bitterness, acid aroma and sour taste (the pattern marked at inner of the graphic). Tempe made from Argentina soybean had higher sensory intensity than from Fresh US IP soybean but showed no certain characteristic was dominant. Tempe made from US IP soybean 1 year old (IP soybean after one year of storage) had higher sour taste and after taste than other soybeans. Tempe made from US No. 1 soybean had a dominant sensory characteristic on sour taste; meanwhile local soybean had the highest characteristic of beany flavor and bitterness than the other soybeans. The strong characteristic of bitterness of local soybean may correlate with the quick growth of molds in local soybean. Subsequent growth of molds will continue the breakdown of protein into smaller substances such as ammonia that contribute to bitterness of tempe.
Microbial changing during fermentation

Analyses of total plate count and lactic acid bacteria after 1 day and 2 day soaking (Table 1.2) confirms that bacteria, especially lactic acid bacteria involve in initial step of tempe fermentation, as shown by the decrease in pH. This step is important in providing acidic condition to inhibit the growth of undesirable microorganism in subsequent step. The soybean has to reach pH 4.5-5.3 before inoculation with tempe starter (Pawiroharsono, 1996). During this soaking process, carbohydrate content decreases and the total soluble solid increases (Arbianto, 1978). After 2 day soaking, the number of total microorganism and total lactic acid bacteria decreased (Table 1.2).

During incubation process, the total lactic acid bacteria remained stable, however, the total plate count increased (Table 1.3). During incubation step, mold is expected to grow knit the bean and turn into tempe. The results show that mold grew during the incubation step.

Table 1.2. Total plate count and total lactic acid bacteria during soaking of boiled soybean

<table>
<thead>
<tr>
<th>Analyses parameter</th>
<th>Soaked soybean</th>
<th>Soaking water</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC (cfu/g or cfu/ml)</td>
<td>4.4x10^6</td>
<td>3.0x10^9</td>
</tr>
<tr>
<td>Total lactic acid bacteria (cfu/g or cfu/ml)</td>
<td>4.3x10^9</td>
<td>5.1x10^8</td>
</tr>
<tr>
<td>pH</td>
<td>5.25</td>
<td>4.84</td>
</tr>
</tbody>
</table>

Table 1.3. Total plate count, total lactic acid bacteria and mold during soaking incubation of tempe

<table>
<thead>
<tr>
<th>Analyses parameter</th>
<th>0 day</th>
<th>1 day</th>
<th>2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC (cfu/g)</td>
<td>2.6x10^6</td>
<td>3.1x10^9</td>
<td>1.2x10^9</td>
</tr>
<tr>
<td>Total lactic acid bacteria (cfu/g)</td>
<td>2.4x10^5</td>
<td>2.6x10^6</td>
<td>7.1x10^6</td>
</tr>
<tr>
<td>Total molds (cfu/g)</td>
<td>&lt;1.0x10^6</td>
<td>5.0x10^7</td>
<td>5.9x10^9</td>
</tr>
</tbody>
</table>

CONCLUSION

The best variety of soybean suitable for tempe making is Fresh US IP soybean due to its highest yield in comparison to other four soybean varieties.
tested. Further more tempe produced from the IP-soybean (new) also had the most likeable sensory quality. On nutritional aspect, however, local soybean had tempe with the highest protein content than that of tempe produced by other varieties of soybean. The lactic acid bacteria involved at initial step of tempe fermentation and decreased the pH of soybean. This bacteria remained stable during main fermentation of tempe.

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