Functional properties of tempe protein isolates derived from germinated and non-germinated soybeans

M Astawan, T Wresdiyati, Subarna, Rokaesih and R M Yoshari
Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia

Email: mastawan@yahoo.com

Abstract. Tempe is an indigenous Indonesian soybean fermented food. Tempe is a source of protein which can be used as a raw material for making protein isolate. In this study, the tempe was produced from Grobogan local soybeans with two types of treatment, germinated and non-germinated. The two types of tempe produced were then processed into protein isolates, namely tempe protein isolate from non-germinated soybeans (NGTI) and tempe protein isolate from germinated soybeans (GTI). This research aimed to measure the functional properties of NGTI and GTI and then compared them to CSI (commercial soy protein isolate). The results of this study showed that the functional properties of GTI and NGTI were not significantly different. Still, GTI had significantly higher values ($p < 0.05$) than CSI on the parameters of water absorption capacity (5.86 and 1.57 g water/g sample), oil absorption capacity (2.48 and 1.19 mL oil/g sample), foam capacity (133.50 and 120.00%), foam stability, emulsion stability, and gel strength. From this study, it can be concluded that GTI and NGTI have good functional properties that have an opportunity to be applied in the food industries as a substitute for CSI.

1. Introduction
Tempe is a traditional Indonesian food made of soybean through a fermentation process using the mold *Rhizopus* spp. The fermentation process changes soybeans into tempe that has a unique taste, aroma, texture, and appearance, which differs from its raw material. The average tempe consumption in Indonesia is 10.1 kg/capita/year [1], contributing to 10% of the total daily protein requirement for Indonesia's 265 million people. Improving the quality of tempe protein needs to be done through efforts such as modifying the raw materials using germinated soybeans. The germination process is an easily applied technology that is also affordable.

The germination process of soybeans can change the complex molecules into simpler ones (making them more easily digested and absorbed by the body) and increase the protein content [2]. The protein content of soybean tempe, germinated soybean tempe, and soybeans are 50.18, 53.37, and 39.8–41.8% (dry basis), respectively [3][4]. Other superiorities of tempe compared to soybeans include higher dissolved solids, dissolved nitrogen, free amino acids, free fatty acids, protein digestibility, protein efficiency ratio, and chemical score [1].

Protein isolates are products obtained through the process of reducing or eliminating the main non-protein components such as water, oil, starch, and other carbohydrates so that the protein content could be increased to 90% or greater [5]. Their high protein content makes protein isolates have a wider range of usage in food and beverage products. Protein isolates have a very important role in creating the functional properties of certain food products [6].
Indonesia’s indigenous protein source that has the potential to be developed into raw material for a protein isolate is tempe. Tempe protein isolate could then be used as a raw material or ingredient in food processing. Therefore, there needs to be a study that measures the tempe protein isolate’s functional properties so that the information could be used by food industries that wish to apply the isolate in making sausages, meatballs, baked goods, ice cream, beverages, etc.

2. Methodology

2.1. Materials
The main material used was the Grobogan variety of local soybeans obtained from Grobogan Regency, Central Java, Indonesia, and a commercial soybean protein isolate that is commonly used in the food industry. Additional materials for making tempe included the *Rhizopus* spp. mold inoculum and polypropylene plastic bags. The chemicals needed to produce tempe protein isolate were n-hexane, distilled water, NaOH 2N, and HCl 2N.

2.2. The germinated soybeans preparation.
Soybeans were sorted and washed to remove any dirt or contaminants and then soaked in water (1:5 w/v) for four hours. Any floating soybeans were discarded. The soybeans were then incubated for 28 hours in the dark. During incubation, the soybeans were watered every four hours to maintain the humidity, resulting in sprouts with a radicle of ± 0.5 cm [7].

2.3. The tempe preparation.
The tempe-making process from soybeans and germinated soybeans was conducted according to the procedures used in Rumah Tempe Indonesia [1]. Soybeans were sorted first and soaked for two hours. And then the soybeans were boiled for 30 minutes, cooled, and soaked for 18–30 hours at room temperature. The soybeans were then milled so that the seeds split, washed, and had the hulls removed. The dehulled soybeans were washed thoroughly, run under hot water, drained, and cooled to room temperature. After they were cool enough, the dehulled soybeans which had been boiled were inoculated with commercial *Rhizopus* spp., inoculum (2 g/kg dry soybeans), packed in 5.5 cm-diameter plastic bags with perforations 2 x 2 cm apart, and then fermented for 48 hours at 30 °C.

2.4. The low-fat tempe flour preparation.
Fresh tempe was sliced and blanched using hot steam for two minutes then dried using a cabinet dryer at 60 °C for eight hours. The dried tempe was ground using a disc mill and sifted using a 60 mesh sieve [8]. The fat content of the tempe flour was lowered by extraction using hexane (tempe flour: hexane = 1:3 w/v) for 2 hours at room temperature using a magnetic stirrer. The sample was then filtered using filter paper (Whatman paper 43) assisted by a vacuum pump. The filtered sample was kept in an acid cabinet and the resulting low-fat tempe flour was dried using a drying oven at a temperature of 50 °C for 2 hours until the solvent evaporated [9].

2.5. The tempe protein isolate preparation.
The low-fat tempe flour from soybeans and germinated soybeans was dissolved in distilled water 1:10 (w/v), had the nitrogen extracted at a base pH for 2 hours with a magnetic stirrer, centrifuged for 10 minutes at a temperature of 4 °C and a speed of 3000 g [30]. The pH of the centrifuged filtrate was lowered to reach its isoelectric point, extracted for 2 hours with a magnetic stirrer, and centrifuged for 10 minutes at a temperature of 4 °C and a speed of 3000 g. The sediment obtained was washed with distilled water at a ratio of 1:1 (w/v), had its pH neutralized to 7.0 using NaOH 2N, and dried using a freeze dryer.
2.6. Analysis
The types of analysis conducted on the tempe protein isolate and commercial soybean protein isolate consisted of water absorption capacity and oil absorption capacity [10], emulsifying capacity and stability [10], foaming capacity and stability [11], and gel strength [4]. The analysis of antioxidant capacity was conducted using the DPPH method and was stated in mg ascorbic acid equivalent antioxidant capacity units (AEAC)/g sample [11, 13]. The analysis of the total phenolics was conducted using the method in [14] which is based on the product of the reaction between phenol and the Folin-Ciocalteu reagent, resulting in blue that is measured for absorbance at a 765 nm wavelength and stated in mg gallic acid equivalent units (GAE)/g sample.

2.7. The experiment design and data analysis
The study was conducted using a completely randomized design, consisting of three treatments namely tempe protein isolate from germinated soybeans (GTI), tempe protein isolate from non-germinated soybeans (NGTI), and a commercial soybean protein isolate (CSI). Observations were made twice, each duplicate. Statistical testing was conducted with ANOVA (Analysis of Variance) followed up with Duncan’s difference test at an α = 0.05 rate.

3. Results and discussion
3.1. Water absorption capacity
Tempe protein isolate from germinated soybeans (GTI) had a water absorption capacity similar to that of the tempe protein isolate from non-germinated soybeans (NGTI). Still, both were significantly higher (p < 0.05) than that of the commercial soybean protein isolate (CSI) (table 1). The water absorption capacity of protein isolates is influenced by its protein content. The higher the protein content, the better its water absorption capacity will be. The germination treatment could improve the soybeans’ protein content, so it produced a better tempe protein isolate. During the germination process, there was an increase in both the content and the quality of the soybean protein, and hydrophilic polysaccharide molecules were broken down, so the interaction between protein and water increased [2]. The fermentation process in the tempe production could have also influenced the water absorption capacity in the GTI and NGTI. In the production of tempe, there was an increase in protein content due to the decrease in the content of the other dissolved components in the soybeans during the boiling process and the consumption of the non-protein compounds by the mold to support its growth during the tempe fermentation process [15].

| Parameters                        | Sample      |
|-----------------------------------|-------------|
| Water absorption capacity (g water/g sample) | CSI 1.57 ± 0.01\textsuperscript{a} GTI 5.86 ± 0.32\textsuperscript{b} NGTI 5.75 ± 0.32\textsuperscript{b} |
| Oil absorption capacity (mL oil/g sample)   | 1.19 ± 0.00\textsuperscript{a} 2.48 ± 0.23\textsuperscript{b} 2.20 ± 0.20\textsuperscript{b} |

\textsuperscript{a}The values on the same line followed by different letters show significantly different results (p < 0.05).

The implication of a better water absorption capacity in the GTI and NGTI is the use of these products would be more effective than CSI to obtain similar characteristics in a certain product. The addition of protein isolates plays a role in the creation of a better texture in meat emulsions and bread doughs and reducing the loss of moisture during the cooking and baking process.
3.2. Oil absorption capacity
There was no difference between the oil absorption capacity between the GTI and NGTI, but both had a significantly higher oil absorption capacity ($p < 0.05$) than the CSI (table 1). This was due to the influence of the fermentation process where the activity of the tempe mold (*Rhizopus* spp.) breaks down the complex proteins in the soybeans into simpler proteins, making the particles smaller and the texture finer and more porous. This condition enables a better oil absorption process. The soybean boiling process in tempe production also causes protein denaturation, which exposes the non-polar (hydrophobic) side of the protein polypeptide. This increases the interaction between protein and oil (non-polar compounds), so more oil is absorbed by the proteins [16].

The germination process in soybeans activates an enzyme system to break down complex molecules such as proteins, carbohydrates, and fats into simpler forms [17], so the GTI particles became smaller and denser. According to [18], the germination process could increase the number of lipophilic proteins, which contribute to the increased fat globule-retention capacity. The high oil absorption capacity in GTI and NGTI also indicated a large number of non-polar (hydrophobic) protein branches in the two protein isolates. This means that GTI and NGTI are very suitable for the development of emulsion products such as sausages, meatballs, ice cream, and so on. The hydrophobic amino acids that are believed to influence oil absorption capacity are alanine, valine, glycine, isoleucine, leucine, phenylalanine, proline, tryptophan, and tyrosine [19].

3.3. Foaming capacity and stability
The results for the foaming capacity analysis are presented in table 2 which revealed that the germination treatment influenced the foaming capacity. The GTI had a foaming capacity that was not significantly different from those of the CSI and NGTI. The strength of the proteins in trapping gas is the factor that determines the character of the foam produced [20]. Protein is absorbed on the surface and forms a stable film surrounding the foam and creates good-quality foam [21].

The foam stability of the three types of protein isolates during the observations at minutes 1, 5, 10, 15, 20, 25, and 30 are presented in figure 1. The NGTI had better foam stability than GTI and CSI, 46.36% up to the 30th minute. The GTI experienced a faster decline in foaming capacity compared to the NGTI. The germination process increases foaming capacity but decreases foam stability [17]. The germination process probably causes denaturation of the protein surface and decreases the surface tension of the molecules that contribute to the foam formation. Germination also causes changes to the protein conformation which possibly affects the stability of foam from legume flours [2].

| Table 2. The foaming and emulsion capacity of isolate protein*. |
|---------------------------------------------------------------|
| **Parameters** | **Sample** | **CSI** | **GTI** | **NGTI** |
| Foaming capacity (%) | 120.00 ± 4.00a | 133.50 ± 14.64ab | 167.00 ± 18.36b |
| Emulsion capacity (%) | 122.00 ± 0.04a | 124.00 ± 0.09a | 119.00 ± 0.06a |

*The values on the same line followed by different letters show significantly different results ($p < 0.05$).
3.4. Emulsion capacity and stability

The results for the emulsifying capacity analysis of the tempe protein isolates are presented in table 2. The results of the statistical analysis revealed that the GTI, NGTI, and CSI had emulsifying capacities that were not dissimilar, even though GTI tended to have a higher emulsifying capacity. Therefore, the germination process in soybeans did not have a significant effect on the emulsifying capacity of the tempe protein isolates produced. The emulsifying capacity of proteins depends on the stability of the hydrophilic and lipophilic bonds [22]. The GTI and NGTI had stable hydrophilic and hydrophobic groups which were indicated by the high water absorption capacity and oil absorption capacity of the two products.

![Figure 1. Foaming stability (%) of isolate protein.](image)

![Figure 2. Emulsion stability of isolate protein.](image)

The germination process probably caused the partial structure of polypeptides to dissociate and open, so the hydrophobic side of the amino acids became exposed. This would help the hydrophobic peptide chain bond with fat droplets. In the end, the process would increase the protein volume or surface area and also improve the emulsifying capacity [17]. During the germination process, the amount of dissolved protein increased. Dissolved protein has a better surfactant nature, which triggers the creation of the oil in water emulsion.

The emulsion stability profiles demonstrated by the three types of protein isolates are presented in figure 2. The stability of the emulsion formed by GTI and NGTI remained whole (100%) until the 90th minute, whereas the stability of the emulsion formed by CSI only remained 91% at the 90th minute. The stability
of the emulsion formed by GTI appeared to be slightly higher than that of the NGTI. The germination process increases emulsion stability by 5–7% [2].

The tempe producing process also increased the emulsifying capacity and stability due to the increased protein content and amount of dissolved protein. The stability of an emulsion depends on the interpartial strength of the material in maintaining the hydrophobic interaction between oil and protein. [23] stated that protein has a stabilizing effect on emulsions by creating a membrane matrix around the oil droplet. This is the reason why the GTI and NGTI had similar emulsion stabilities.

3.5. Qualitative gel strength

A qualitative analysis of the gel strength was conducted in a tube. The results of the gel strength analysis of the three types of protein isolates are presented in table 3. The higher the concentration of the CSI, GTI, and NGTI samples, the more readily the gel is formed. In GTI, a concentration of 12.5% could form a very strong gel which was not dislodged when the tube was inverted vertically and jolted more than five times. At a concentration of 12.5%, CSI could not even form a gel while NGTI could form a gel, but the gel was dislodged when the tube was inverted vertically and jolted more than once.

| Sample concentration % (b/v) | Qualitative observation |
|-------------------------------|-------------------------|
| CSI                          | GTI                     | NGTI          |
| 7.5                          | 0                       | 0             | 0             |
| 10.0                         | 0                       | 0             | 0             |
| 12.5                         | 0                       | 4             | 3             |
| 15.0                         | 1                       | 4             | 4             |

Notes:
0 = gel not formed.
1 = gel very weak, gel dislodged when the tube was tilted.
2 = gel was not dislodged when the tube was inverted vertically.
3 = gel was not dislodged when the tube was inverted vertically and jolted once.
4 = gel was not dislodged when the tube was inverted vertically and jolted > 5 times.

The difference in the protein content between protein isolates influenced the gelling results. The higher the protein concentration in the protein isolate, the stronger the gel formed tended to be. Protein concentration was the factor that affected the gelling capability due to a heating process [24]. The boiling treatment undergone by the soybeans during the tempe-making process caused the protein to denature and increased the protein concentration [25]. The protein denaturation during the tempe-making process in the GTI and NGTI increased their gel strength.

3.6. Antioxidant capacity and total phenolic content

The results of the antioxidant capacity analysis are presented in table 4. The GTI had a significantly higher antioxidant capacity (p < 0.05) than that of either the NGTI or the CSI. The antioxidant capacity of the NGTI was also significantly higher than that of the CSI. This could be due to two important treatments in the tempe-making process, the soybean germination process and the soybean fermentation process. During the soybean germination process, the phenolic content and vitamin E content are increased [26]. During the soybean fermentation process to make tempe, the phenolic compound content increased, which led to increased antioxidant capacity. Tempe's antioxidant activity is supported by its phenolic components [26].
Table 4. Analysis of antioxidant capacity and total phenol levels of protein isolates*.

| Parameters                        | Sample       |
|-----------------------------------|--------------|
|                                   | CSI          | GTI          | NGTI         |
| Antioxidant capacity              |              |              |              |
| (mg AEAC/g sample db)             | 26.02 ± 0.72a| 171.91 ± 11.48b| 157.79 ± 4.80b|
| Total phenolic content            | 368.70 ± 10.55a| 1142.41 ± 1.91c| 1020.39 ± 8.6b|

* The values on the same line followed by different letters show significantly different results (p < 0.05).

The results of the total phenolic content analysis of the protein isolates are presented in table 4. The GTI had a significantly higher (p < 0.05) total phenolic content than the NGTI and the CSI. The total phenolic content of the NGTI was also significantly higher than that of the CSI. The germination process could increase the total phenolic content due to the synthesis of phenolic compounds. Seeds synthesize phenolic compounds during the initial phase of germination to protect the growth of the hypocotyl from oxidative reactions caused by environmental influences.

In comparison to the dormant phase, germinated soybeans would undergo an increase in the total phenolic content and antioxidant activity by 201 and 175%, respectively [26]. The phenolic content of legumes has a positive correlation with their antioxidant capability. However, the non-phenolic contents such as ascorbic acid, tocopherol, phytic acid, carotenoid, and saponin are also believed to play a role in antioxidant activity [28]. Also, the heating process during tempe production could also increase the total phenolic content and radical scavenging activity [29].

4. Conclusion

The results of the analysis of the functional properties of protein revealed that protein isolates from germinated soybean tempe (GTI) was superior to that of the non-germinated soybean tempe (NGTI). When compared to a commercial soybean protein isolate (CSI), the GTI had better protein functional properties in terms of water absorption capacity, oil absorption capacity, foaming capacity and stability, emulsifying stability, and gel strength. Therefore, the GTI has great potential to be applied in the food industry to improve the physical characteristics of various foods and beverages.

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