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FULL PAPER

Application of ultrasound in germinated soybean tempe protein concentrate production with various types of solvents

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Abstract

Tempe is a traditional Indonesian fermented food that has high protein content. It has been known as a source of bioactive compounds, such as peptides and isoflavones, which contribute to immune health. Therefore, tempe has the potential to be processed into raw materials for food and supplement products, such as protein concentrate. In this study, the tempe was made using germinated and non-germinated soybeans. Tempe protein concentrate production process was carried out by removing the fat in tempe flour by conventional and ultrasound-assisted extraction methods using two types of solvents: ethanol and hexane. This research aimed to compare the physicochemical characteristics of the protein and concentrates on obtaining the optimum treatment with the highest protein content. The results showed that ultrasound-assisted extraction using hexane significantly improved fat extraction, resulting in protein concentrate with higher protein content. Germinated soybean tempe protein concentrate extracted using hexane had the best protein concentrate, which consisted of the highest protein content (72.8%), brightness value (L*) 84.3, whiteness index 81.3, bulk density 0.5 g/mL, and angle of repose of 29.4°. The germination process in soybean improved the protein content of the concentrate as the result of other components' degradation into energy required for new protein synthesis.

1. Introduction

Tempe is a traditional Indonesian food from soybean that undergoes a fermentation process with the *Rhizopus* spp. mould. The fermentation by *Rhizopus* spp. resulted in several enzymes that can hydrolyse complex compounds in the soybean into simpler compounds in the tempe to be easily digested and absorbed by the body (Kadar *et al*., 2018). After the fermentation, there is an increase in the free amino acid content of 7.3–30% as a result of the proteolytic enzyme activity caused by the mould. This condition resulted in the tempe being known as a plant-based food rich in protein and having high-quality nutrition (Puteri *et al*., 2018). In addition, Tempe is rich in bioactive components, such as peptides and isoflavones, which have roles in health and immunity systems (Astawan, Rahmawati, Cahyani *et al*., 2020). However, tempe is classified as food with a short shelf-life (1–2 days at room temperature); thus, it needs further processes to extend its shelf-life (Astawan *et al*., 2016).

It is also a product with a minimum protein concentrate content of 65%. The higher protein content can be achieved by reducing fat content, partial reduction of carbohydrates and minerals, and other components (CAC, 1989). The protein concentrate can be utilised in bakery products, processed meats, dairy products, and supplements (Berk, 1992). The raw materials used in this study were tempe flour made from germinated and non-germinated soybeans. The soybean germination process was proven to increase the protein digestibility value, total phenolic content, and reduction of phytic acid in the soybean (Jiang *et al*., 2013; Astawan, Wresdiyati, Subarna *et al*., 2020) and increase antioxidant capacity in soybean and mung bean (Huang *et al*., 2014).

In this study, the process of fat reduction in tempe flour was performed using two types of solvents, hexane and ethanol, and two extraction methods, which were conventional and ultrasound-assisted extraction. Ultrasound was proven to increase the extraction capacity as the effect of cavitation phenomena, which form sound waves that could break down the cell biomass and decrease its particle size (resulting and Rathod, 2013). The resulted soybean tempe protein concentrates were compared to determine the effects of solvent types, extraction methods, and soybean

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germination process on the yield and physicochemical characteristics of tempe.

2. Materials and methods

2.1 Soybean germination

Local soybeans (*Glicine max* L.) grobogan varieties were purchased from farmers in Grobogan, Central Java, Indonesia. The soybean germination process was performed according to the method described by Puteri *et al*. (2018). The soybean was sorted and cleaned, then soaked in water with a 1:5 (b/v) ratio for 3 hrs. After being soaked, the soybean was incubated for 28 hrs in dark conditions and watered every 4 hrs until a radicle of 0.5 cm long sprouted from the germinated soybean was obtained.

2.2 Tempe production

The tempe production method was referred to as the standard method of Rumah Tempe Indonesia (Astawan, Wresdiyati, Subarna and Asyaifullah, 2020). The tempe production from germinated and non-germinated soybean was carried out separately. The soybean was sorted, soaked for 2–4 hrs, boiled for 30 mins, re-soaked for 15–20 hrs until the pH level reached 3.5–5.2, dehulled, cleaned and rinsed with hot water, drained, inoculated with 0.2% yeast (w/w from dry soybean), packed in perforated PP plastic, and fermented for 40 hrs. Similar processes were also done in germinated soybean, but the tempe production step from the germinated soybean production process was started by boiling for 30 mins.

2.3 Tempe flour production process

The tempe flour production process was performed according to the method described by Puteri *et al*. (2018). Fresh tempe from the prior process was sliced using a slicer (R 6-7 Sweden) with 0.5 cm thickness, then blanched using a steamer (1 bar) for 2 mins, dried with a cabinet dryer (6073 Dreich, West Germany) at 60°C for 8 hrs. Then, the dried tempe was milled using a pin disc mill (Alecanderwerk Inc., Germany) with a 60-mesh sieve.

2.4 Tempe protein concentrate production

The fat extraction of tempe flour was conducted according to Wang *et al*. (2004) with slight modifications. In the conventional extraction method, the maceration was performed twice, each for 1 hr, with a flour and solvent ratio of 1:3 (b/v). The solvents used for the extraction were ethanol and hexane. Then, the filtration process using a vacuum pump was done to achieve low-fat tempe flour. The flour was dried at room temperature for 12 hrs, then dried using a drying oven at 55°C for 4 hrs. The low-fat tempe flour from conventional extraction was re-extracted using the ultrasound method (Branson Ultrasonics Corporation, USA) with hexane and ethanol solvents in the ultrasound -assisted extraction. The ratio between flour and solvent was 1:4 (b/v). The sonication was conducted for 20 mins at room temperature. Then, the filtration process was carried out using a vacuum pump to obtain the protein concentrate. The solids were air-dried at room temperature for 12 hrs to remove the solvent residues and then dried using a drying oven at 55°C for 4 hrs.

2.5 Yield and proximate analysis

The yield percentage was calculated based on the weight of the tempe protein concentrate divided by dry soybean weight as the raw material in tempe production. The proximate analysis referred to the AOAC (2012) method, which consisted of water (AOAC 925.09) and ash content (AOAC 923.03) determined by the gravimetric method, fat content (AOAC 922.06) determined by Soxhlet method, protein content (AOAC 955.04D) determined by the Kjeldahl method, and carbohydrate content, which was calculated by difference.

2.6 Physicochemical characteristic analysis

2.6.1 Colour and whiteness index analysis

The colour and whiteness index (WI) were determined according to the method described by Hunter (1975). The analysis was performed using chromameter (Konica Minolta CR-310). The data obtained from the colour measurement were Hunter L, a, and b. The chromameter analysis could be used to determine the whiteness index with a formula as followed:

$$WI = 100 - \sqrt{(100 - L)^2 + (a^2 + b^2)}$$

where *L* is brightness value (0-100), *a* is red-green chromatic colour, and *b* is blue-yellow chromatic colour

2.6.2 Bulk density analysis

Bulk density was determined by the method described by Okezie and Bello (1988). The protein concentrate sample was poured into a 10 mL beaker glass that had been determined for its weight. The glass-filled sample was tapped >30 times, so no cavities were found when the sample was tared until 10 mL. The beaker glass weight was measured. The bulk density resulted from sample weight (g) divided by its volume (mL).

2.6.3 Angle of repose analysis

The angle of repose analysis was measured by pouring the flour at a certain height aided with a cone on

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a flat surface. The angle of repose was determined by calculating the base diameter (d) and height of the flour (h). The angle of repose was calculated by the equation as followed:

$$\text{The angle of repose} = \text{arc tan}\,\frac{2h}{d}$$

2.7 Research design

This study used a factorial design with two factors and two levels. The first factor was tempe flour (A) with two levels, A1: Tempe flour from germinated soybean and A2: Tempe flour from non-germinated soybean. The second factor was the type of solvents (B) with two levels, which were B1: hexane and B2: ethanol. Based on this research design, there were four treatment combinations with two repetitions each. The data were analysed using ANOVA (analysis of variance), followed by the Duncan Multiple Range Test with a 95% confidence level.

3. Results and discussion

3.1 The effects of ultrasound during the extraction process

Figure 1 shows that ultrasound-assisted extraction (UAE) produced protein concentrate with higher protein content than the conventional method. The highest protein content was obtained from a combination of tempe flour produced from germinated soybean extracted using hexane as the solvent, with a protein content of 72.78%. This can be caused by the capacity of ultrasonication to remove oil from the substance, resulting in an increase in protein content in the material. The cavity phenomena generated by ultrasound during the extraction process, resulting in smaller flour particles. The smaller size resulted in contact area expansion with the solvent (Zhao *et al.,* 2022). The sonication process changes the structure of plant tissue through destruction, increased porosity, and intercellular distances. This lowers the resistance to mass transfer from the solute, allowing more solvents to penetrate the plant tissue (Frohlich *et al.,* 2022). Ultrasonic cavitation also promotes the release of oil from the extracellular matrix, allowing extraction to be performed under mild processing conditions (Thilakarathna *et al.,* 2022). Therefore, during the extraction process using ultrasound, the fat was effectively extracted as compared to the conventional method.

The ultrasound method had been widely applied in fat extraction studies of various plant materials, such as seeds (Zhong *et al.,* 2018; Song *et al.,* 2019; Santos *et al.,* 2021), husk (Chen *et al.,* 2014), leaves (Palsikowski *et al.,* 2020; Frohlich *et al.* 2022), to microalgae (Menéndez *et al*., 2014), in which ultrasound-assisted extraction was more efficient than the conventional methods. Therefore, the protein concentrate observed in this study was the protein concentrate obtained from the ultrasound-assisted extraction method instead of the conventional extraction approach.

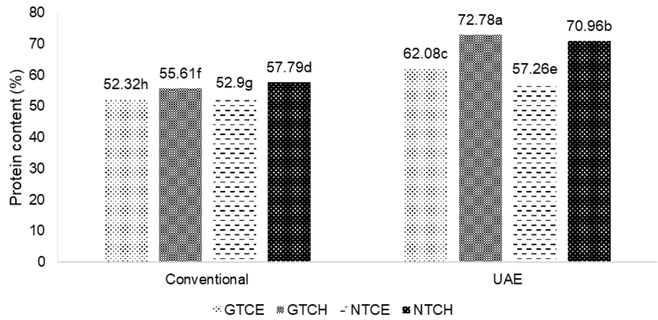

Figure 1. Effect of ultrasound-assisted extraction on the protein content of various tempe protein concentrate. Values with different letters are statistically significantly different (p<0.05). GTCE: Germinated soybean tempe protein concentrate extracted using ethanol, GTCH: Germinated soybean tempe protein concentrate extracted using hexane, NTCE: Non-germinated soybean tempe protein concentrate extracted using ethanol, NTCH: Non-germinated soybean tempe protein concentrate extracted using hexane

3.2 Yield and proximate analysis

The yield data of tempe protein concentrates is shown in Table 1. The germination process had a significant effect (p<0.05) on the tempe flour yield as the result of the water imbibition into the soybean during the germination process. Hence, the water content in the tempe increased (Ojha *et al*., 2014), and then the water evaporated during the drying and tempe milling process. The variance analysis showed that the soybean germination process and solvent type had a significant effect (p<0.05) on tempe protein concentrate yield. However, the interaction between both factors (soybean germination and solvent types) had no significant effect (p>0.05) on the protein concentrate yield. The tempe protein concentrates with hexane as the solvent had a lower yield compared to tempe protein concentrate treated with ethanol. The hexane capacity in extracting fat is more effective than ethanol (Aziz *et al*., 2009), there was more fat extracted and removed from the tempe flour along with hexane. The difference in the

Table 1. Effect of germination process and types of solvents on yield of tempe protein concentrate

Yield (%)	Germinated soybean	Non-germinated
Tempe flour	59.5 ± 1.2^{b}	66.5 ± 1.3^{a}
Tempe protein concentrate		
a. Ethanol	49.7 ± 1.1^{b}	56.3 ± 0.0^{a}
b. Hexane	43.8 ± 0.9^{a}	49.4 ± 0.1^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different (p<0.05).

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protein concentrate yield was also affected by the variations in the resulted tempe flour.

The result of tempe protein concentrate proximate analysis with two different solvents is shown in Table 2. The result showed that the soybean germination process had a significant effect ($p<0.05$), but the solvent type had no significant effect ($p>0.05$) on the tempe protein concentrate water content. The final product contains varying protein contents depending on the solvent used. The capacity of each solvent to aggregate protein and remove fat and water varies based on the polarity. The protein and fat content of the protein concentrate produced will be affected by this condition. Because of its non-polar properties, hexane could extract fat more efficiently than ethanol as a semi-polar solvent (Zulqarnain *et al.,* 2021). This is in accordance with some studies described by Arguelles-Peña *et al.* (2021), Hasibuan and Gultom (2021), Oladipo and Betiku (2019), and Perrier *et al.* (2017), which showed the capability of hexane in resulting highest yield of oil extract in moringa seeds, lime peel, and rapeseed flakes. The non-polar groups (i.e. fatty acid) of the triglycerides may have reduced the solubility of ethanol as a polar solvent, resulting in lesser oil extraction. Furthermore, the increased oil removed with hexane might be caused by the absence of the OH group, which has been shown to interfere with various oilseed extraction processes (Oladipo and Betiku, 2019). Moreover, the germination process also affected the protein content in the concentrate as the result of other component degradation, such as fat and carbohydrate into energy needed for new protein synthesis (Shi *et al*., 2010).

The germination process and solvent type also significantly affected the fat content in the tempe protein concentrate ($p<0.05$). In the soybean germination process, the fats were degraded into free fatty acids, which quickly dissolved the fat during the extraction process. This condition resulted in the low fatty acid of the germinated tempe concentrate. The extraction with hexane also resulted in tempe protein concentrate with lower fat content than tempe protein concentrate with ethanol as the solvent. The non-polar properties of fat made it easy to extract with hexane which also had similar non-polar properties (Wildan *et al*., 2013). The low-fat content in the GTCH (germinated soybean tempe protein concentrate extracted using hexane) was following the low yield of GTCH. This condition was induced by the removal of fat from tempe flour during the manufacturing of tempe protein concentrate.

The ash content in the tempe protein concentrate was also affected by the solvent type during fat extraction. Hexane resulted in tempe protein concentrate with higher ash content compared to ethanol. According to Bintanah and Handarsari (2014), the minerals in tempe were mostly polar, which causes more minerals to be extracted or removed when using ethanol as a solvent compared to hexane. The solvent type also significantly ($p<0.05$) affected the carbohydrate content in hexane-treated tempe protein concentrate. The carbohydrate content in the tempe protein concentrate extracted using ethanol was lower compared to tempe protein concentrate extracted using hexane. Because ethanol and carbohydrate have comparable polar properties (Sani *et al.,* 2013), more carbohydrates in tempe flour were extracted.

3.3 Physicochemical characteristics

3.3.1 Colour and whiteness index

Colour is one of the most critical parameters in determining food quality. Colour is strongly associated with physical characteristics, chemical properties, and sensory indicators of food material (Mendoza *et al*., 2006). The colour of protein concentrate with less dark colour enables the broad application of protein concentrate. The appearance of tempe protein concentrate is shown in Figure 2, while the result of colour analysis is shown in Table 3. The soybean germination process and solvent types had significant effects ($p<0.05$) on the brightness value (L*) of the tempe protein concentrate. The lightness also strongly affected the whiteness index of the tempe protein concentrate. The higher the brightness value, the whiteness degree became higher as well. The increased

Table 2. Effect of germination process and types of solvents on proximate of tempe protein concentrate.

Parameter	Tempe Protein Concentrate			
	GTC		NTC	
	Ethanol	Hexane	Ethanol	Hexane
Moisture (%wb)	5.3±0.3^{ab}	5.7±0.0^{b}	5.0±0.2^{a}	5.2±0.1^{a}
Ash (%db)	1.9±0.1^{a}	2.5±0.2^{b}	2.0±0.0^{a}	2.5±0.8^{b}
Fat (%db)	18.0±0.0^{b}	2.7±0.1^{a}	20.2±0.4^{c}	2.9±0.2^{a}
Protein (%db)	62.1±1.4^{b}	72.8±0.6^{c}	57.3±0.2^{a}	71.0±0.2^{c}
Carbohydrate by difference (%bk)	12.7±1.2^{a}	16.4±0.5^{b}	15.5±0.4^{ab}	18.4±0.4^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). GTC: Germinated soybean tempe protein concentrate, NTC: Non-germinated soybean tempe protein concentrate.

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Table 3. Effect of germination process and types of solvents on physicochemical characteristics of tempe protein concentrate.

Parameter	Tempe Protein Concentrate			
	GTC		NTC	
	Ethanol	Hexane	Ethanol	Hexane
L	72.7±0.9[a]	88.3±0.4[c]	72.2±0.2[a]	80.1±0.5[b]
a	0.4±0.4[ab]	-0.2±0.1[a]	0.6±0.1[b]	-0.1±0.2[a]
b	15.8±0.0[b]	10.1±0.7[a]	16.0±0.6[b]	9.7±1.1[a]
Whiteness Index (%)	68.5±0.2[b]	81.3±0.6[c]	68.0±0.4[a]	77.8±0.4[b]
Bulk density (g/mL)	0.5±0.0[a]	0.5±0.0[a]	0.5±0.0[a]	0.5±0.0[a]
Repose angle	40.2±0.2[b]	29.4±1.8[a]	41.6±0.2[b]	33.3±0.3[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). GTC: Germinated soybean tempe protein concentrate, NTC: Non-germinated soybean tempe protein concentrate.

brightness was caused by the reduced fat content after the extraction (Rieuwpassa *et al*., 2013), along with the dissolved pigments of tempe flour by the solvent during the extraction process. The ANOVA result showed that the soybean germination process and solvent type had significant effects on the whiteness index of the tempe protein concentrate. The soybean germination process affected the stability of natural soybean pigments; thus, it affected the protein concentrate colour of the germinated soybean tempe. In hexane-treated tempe protein concentrate, the brightness value and whiteness index results were higher compared to ethanol-treated tempe protein concentrate. Hexane had a polarity index of 0, which made it dissolve lipophilic compounds, such as wax, colour pigments, sterol, and several types of terpenoids and alkaloids (Yusnawan, 2013).

Figure 2. Physical appearances of various tempe protein concentrate. GTCE: Germinated soybean tempe protein concentrate extracted using ethanol, GTCH: Germinated soybean tempe protein concentrate extracted using hexane, NTCE: Non-germinated soybean tempe protein concentrate extracted using ethanol, NTCH: Non-germinated soybean tempe protein concentrate extracted using hexane

The solvent types had significant effects ($p<0.05$) to red-green ("a") and blue-yellow ("b") chromatic colours in tempe protein concentrate. The negative "a" value in hexane-treated tempe protein concentrate was caused by the dissolved carotene pigment in a non-polar solvent such as hexane (Arifulloh *et al*., 2016). The reason was the lower "b" value in hexane-treated tempe protein concentrate, which indicated that the protein concentrate colour was no more yellow than protein concentrate with ethanol solvent.

3.3.2 Bulk density

Bulk density is a particle mass that occupies a specific unit volume. The higher the bulk density, the denser the product becomes. The bulk density calculation is related to the packing and storing of food material (Rohmah, 2012). The tempe protein concentrates bulk density data is shown in Table 3. The result showed that the germination process and solvent type had no significant effects on the bulk density. Powdered-form food material usually had a bulk density of 0.3–0.8 g/mL (Wirakartakusumah *et al*., 1992). Therefore, all tempe protein concentrate samples in this study had the appropriate bulk density result.

3.3.3 Angle of repose

The angle of repose of tempe protein concentrate can be seen in Table 3. The ANOVA result showed that solvent types strongly affected ($p<0.05$) the angle of repose of tempe protein concentrate, but the soybean germination process had no significant effect. The angle of repose from hexane-treated protein concentrate showed the tendency to be smaller than the angle of repose of ethanol-treated protein concentrate. This result was caused by different components in each protein concentration. In hexane-treated protein concentrate, the fat content was lower compared to ethanol-treated protein concentrate. The higher fat content made the protein concentrate particles cohesive. A material that had a higher static coefficient of friction would result in a higher angle of repose. A low angle of repose indicated an improved flour flow index (Anwar and Jufri, 2010). A material that easily flowed had an angle of repose of less than about 40^o (Jan *et al.,* 2016). The result showed that GTCH fulfilled the requirements of free-flowing material with the angle of repose result approximation of 29.40°. Flow properties play a significant role in protein concentrate processing and handling, such as mixing, storage, packaging, transportation, and compression (Barretto *et al.,* 2021). Therefore, the better the flow properties, the easier it will be to handle.

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4. Conclusion

Ultrasound-assisted extraction significantly improved fat extraction in tempe protein concentrate production. The soybean germination process and the used solvent in the extraction process significantly affected the chemical compositions and physicochemical characteristics of tempe protein concentrate. Hexane was the best solvent for tempe protein concentrate with the highest protein content, whiteness index, and a lower repose angle than ethanol. The germination process significantly increased the protein content and whiteness index so that the GTCH could be utilised as raw material with high protein content with no significant colour change.

Conflict of interest

The authors declare no conflict of interest.

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