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Characterization of biochemical and functional properties of water-soluble tempe flour

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Abstract

Characteristics of water-soluble flours from soy (SF), soy tempe (STF), and germinated-soy tempe (GTF) were compared with those of commercial soy protein isolate (SPI). Defatted flour of soy, soy tempe, and germinated soy tempe were extracted in alkaline water (pH 9) and freeze dried to produce water-soluble flours. Protein contents of SF, STF, and GTF were 49%, 47%, and 51%, respectively, and lower than that of SPI (84%). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles showed that STF and GTF contained lower molecular size of proteins compared to SF and SPI. Trypsin inhibitor activity was detected only in SPI. The most abundant phytic acid was contained in SF, followed in order of decreasing abundance, by SPI, STF, and GTF. Antiradical activities measured by DPPH assay also showed significant variations, and the activity was highest in GTF, followed in order of decreasing activities, by STF, SF, and SPI. The foaming and emulsion capacities of STF and GTF were significantly lower than SPI, but higher than SF. These data strongly suggest that STF and GTF have better functional characteristics than commercial SPI. However, optimization of the extraction process is needed to improve the yield and protein content.

Keywords: protein; soybean; germinated-soy; soy protein isolate; foaming and emulsion properties.

Practical Application: Producing food ingredient based on fermented soybean (tempe).

1 Introduction

Tempe is popular fermented food in Indonesia. The health benefits of tempe have been reported (Astuti et al., 2000). Zhan & Ho (2005) reported that tempe could significantly reduce total cholesterol, LDL, and triglycerides content in blood. Tempe was also reported to have a hepatoprotective effect (Mohd Yusof et al., 2013), ACE-inhibitory and antioxidant activity (Gibbs et al., 2004), and immunological impact on intestinal mucosa (Soka et al., 2014).

Tempe is also known to contain high quality and high digestibility of protein. Mice fed with tempe flour showed significantly higher feed conversion ratio and true protein digestibility than mice fed with soy flour (Astawan et al., 2015). Soybean as the raw material of tempe is rich in essential amino acids compared to other plant protein sources (Soares et al., 2005). During fermentation, the quality of the protein is improved. Partially hydrolyzed protein with high digestibility is produced and allergenicity of the protein is reduced after fermentation (Chang et al., 2009; Wilson et al., 2005). The antinutrient compounds of soybean was also reduced during tempe processing (Haron & Raob, 2014).

Tempe has a short shelf life due to continuous fermentation which may lead to discoloration and production of unpleasant flavor from ammonia (Nout & Kiers, 2005). Several studies related to this problem have been released, such as innovation in extending tempe shelf life by combining steam blanching at 80 °C (three minutes) and vacuum packaging (Astawan et al., 2016a) and tempe flour production. However, the application of tempe flour has been limited. Processing tempe into water-soluble flour for food ingredient can be an alternative approach to expand its utilization.

Production of water-soluble flour based on tempe might improve the utilization of protein in tempe. Water-soluble flour of tempe might be applied widely as a food ingredient. It can be used as a protein source or applied to improve the functionality of foods. Zayas (2012) reported that polypeptides in a smaller size can provide better functionality on the food system. Water-soluble flour based on germinated-soy tempe is also an interest to be analyzed. Zieliński (2003) reported that germination increased the protein content of soybean, while carbohydrate and lipid contents were reduced (Shi et al., 2010). Our previous study also found that germinated-soy flour had better antioxidant activity and functionality than soy flour (Astawan & Hazmi, 2016). This research was aimed to study the characteristics of water-soluble flour of tempe (made of soy and germinated-soy) and to compare them with those of commercial soy protein isolate and water-soluble flour of soy.

2 Materials and methods

Whole soybean seeds (var. Grobogan) were obtained from Grobogan, Central Java, Indonesia. Commercial soy protein isolate (ISP-YX 2000, Shandong-Yuxin, Bio-tech Co.Ltd., China)

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was used to compare the products. Commercial powdered tempe starter (RAPRIMA, PT Aneka Fermentasi Industri, Bandung, Indonesia) for tempe production was purchased from Rumah Tempe Indonesia, Bogor, Indonesia. All other chemicals used in the analyses were of analytical grade.

2.1 Water-soluble flour processing

Preparation of tempe

Production of tempe was carried out in Rumah Tempe Indonesia, Bogor by the standard method of the company. Soybeans were sorted, soaked for 2 hours in water, boiled for 60 min then soaked for 24 hours, dehulled, poured by water (100 °C), drained, inoculated by starter (15 g per 10 kg soybeans), packed in perforated polypropylene bags (25 cm x 12 cm), and fermented for 40 hours at 30 °C. For germinated-soy tempe, soybean was germinated before processed into tempe. Soybeans were soaked in water at room temperature (27 °C) for 3 hours. Following the draining process, soybeans were then moved into a perforated container and watered (1:5 w/v) every 4 hours for 20 hours at room temperature to allow the radicle to grow between 0.5 and 2.5 mm.

Preparation of defatted flour

Soybean, soy tempe, and germinated-soy tempe were processed into flour based on the method of Omosebi & Otunola (2013) with slight modifications. Soybeans were dried in a cabinet dryer (Engineering & Equipment GmbH, 6072 Dreieich, West Germany) at 60 °C, and milled by a disc mill with 60 mesh sieve. Soy tempe and germinated-soy tempe were initially sliced to a thickness less than 0.5 cm by a slicer (ALEXANDERWERK, UC II, Montgomeryville, Pennsylvania) and steamed for 2 min before dried in a cabinet dryer at 60 °C. Dried soy tempe and germinated-soy tempe were then milled by a disc mill with 60 mesh sieve. The flours of soy, soy tempe, and germinated-soy tempe were then suspended in *n*-hexane (1:3 w/v) and stirred for 1 hour at room temperature. The solvent was then separated from the precipitate. Following two times of extraction, the precipitate was left in a fume hood to vaporize the solvent.

Extraction

Defatted flour was suspended in water (1:10 w/v) and the pH was adjusted to 9 by using 10 N NaOH. The solution was then stirred for 18 hours at 25 °C and centrifuged for 30 min (BECKMAN, J2-MC, Minnesota, rotor JA-14) by 9000 rpm at 4 °C. The supernatant was collected and dried by a vacuum freeze dryer (EYELA, FD-550, Tokyo Rikakikai Co.,Ltd., Tokyo, Japan). The flour was stored at 4 °C for analyses.

2.2 Chemical composition and functional properties

Proximate composition

Sample chemical compositions were determined by proximate analysis according to the method of AOAC (2012). Moisture and ash contents were determined by the gravimetric method (AOAC 925.09 and AOAC 923.03). Protein content was measured by the Kjeldahl method (AOAC 955.04D). Fat content was determined by Soxhlet method (AOAC 922.06). Carbohydrate content was calculated by difference.

Molecular weight profile

Molecular weight of the protein was estimated using the glycine-SDS-PAGE (14.5% gel) based on the method of Laemmli (1970) and tricine-SDS-PAGE based on the method of Schägger (2006). Sample (50 µL) was prepared in a 50 µl buffer (0.01 M Tris-HCl pH 6.8; 0.1% SDS; 0.1% 2-mercaptoethanol) and kept in water bath (100°C) for 5 min. Precision Plus Protein 10-250 kDa (All Blue Standards, BioRad, Hercules, California) and Natural Polypeptide SDS-PAGE Standards 1.4-26.6 kDa (BioRad, Hercules, California) were used as marker for glycine-SDS-PAGE and tricine-SDS-PAGE, respectively. Coomassie brilliant blue R250 (0.25 g) in acetic acid (10 mL) and methanol solution (90 mL) was used as staining solution.

Trypsin inhibitor activity

The trypsin inhibitor activity was analyzed following the method of Hummel (1959). Trypsin (porcine pancreas) and TAME (*p*-toluenesulfonyl-L-arginine methyl ester) purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) were used as the enzyme and substrate, respectively. The sample was dissolved in a 10 mM Tris-HCl buffer (pH 8) at a concentration of 1 mg/mL buffer. The trypsin was also dissolved in the same buffer at a concentration of 1 mg/ml buffer. Before measurement, the sample solution and the trypsin solution were mixed at a ratio of 1:1 (v/v) and incubated for 5 min at 30 °C Substrate solution made of 1.2 ml Tris-HCl buffer, 1.8 mL TAME (1.74 mM), and 3 µl $CaCl_2$ (1 M) was also incubated for 5 min at 30 °C. For measurement, 5 µL solution of the sample-trypsin solution was added into the substrate solution, and the mixture was kept at 30 °C. The absorbance (247 nm) at just after mixing (0 min) and at 3 min after the mixing was recorded. The trypsin activity of the sample was obtained by the Equation 1:

$$Trypsin\,activity\ (\%) = \frac{Abs\ at\ 3\ min - Abs\ at\ 0\ min}{\Delta t} \times \frac{1000}{2.5} \quad (1)$$

where Δt is the time difference in recording absorbance (3 min).

Phytic acid content

Phytic acid content was measured by colorimetric method based on Lai et al. (2013). Sample (5 g) was extracted in 2.4% HCl (100 mL) and centrifuged (EPPENDORF, 5810 R, Germany) at 3000 rpm for 30 min at 25 °C. The supernatant (3 ml) was mixed with 1 mL of the Wade reagent (0.03% solution of $FeCl_3 \cdot 6H_2O$ containing 0.3% sulfosalicylic acid) and centrifuged at 3000 rpm for 30 min at 25 °C. The absorbance of the mixture was measured with a spectrophotometer (THERMO FISHER SCIENTIFIC, 4001/4, Waltham, Massachusetts) at 500 nm. The mixture of 1 ml Wade reagent and 3 ml distilled water was used as the blank. The concentration of the phytic acid was calculated by a standard curve method.

Antioxidant activity (DPPH assay)

Based on the method of Barreira et al. (2008), 150 µL sample solution (10 mg/ mL) was mixed with a 1 mL DPPH (Wako Pure Chemical Industries, Ltd., Osaka, Japan) solution (2.7 mg DPPH in 100 mL methanol) and incubated in a dark room for 20 min at room temperature. The mixture was then centrifuged with the speed of 10000 × g for 5 min (4 °C using centrifuge (KOKUSAN, H-200, Japan). The absorbance at 517 nm was taken. The antiradical activity to DPPH was calculated using the Equation 2:

$$\text{Antiradical activity on DPPH (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (2)$$

Foaming properties

The foaming capacity and stability were measured by the method of Klompong et al. (2007). The sample solution (60 mL, 3% w/v) was homogenized with a blender (MIYAKO, BL-101 PL, Indonesia) for 1 min. The solution was then placed in a 100 mL graduated cylinder and the volume of the foam was recorded. The foaming capacity was expressed using Equation 3:

$$\text{Foaming capacity (\%)} = \frac{V_1\ (\text{ml}) - V_0\ (\text{ml})}{V_0\ (\text{ml})} \times 100 \quad (3)$$

where V_0 = initial volume and V_1 = volume after homogenization. In addition, the foam stability was obtained as a percentage of the volume of retained foam after 20 minutes (Yin et al., 2008).

Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were measured by the method of Klompong et al. (2007). As much as 300 mg sample was solved in 30-mL distilled water. The solution was then mixed with olive oil (10 mL) and homogenized by using blender with the speed of 20000 rpm for 1 min. Immediately after homogenization, 50 µL emulsion in the bottom of the container was taken and mixed with 5 mL sodium dodecyl sulfate (SDS) 0.1%. The absorbance at 500 nm was measured by a spectrophotometer (THERMO FISHER SCIENTIFIC, 4001/4, Waltham, Massachusetts). The EAI was calculated using Equation 4:

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{protein (g)}} \quad (4)$$

while the ESI using Equation 5:

$$\text{ESI (min)} = \frac{A_{10} \times \Delta t}{\Delta A} \quad (5)$$

where A_0 = absorbance after homogenization; A_{10} = absorbance at 10 min after homogenization; Δt = 10 min; and $\Delta A = A_0 - A_{10}$.

Protein digestibility (in vitro)

Determination of protein digestibility (*in vitro*) was conducted by using pepsin and pancreatin from Sigma-Aldrich Co.LLC Abdel-Aal (2008). Sample solution (1.5 g/30 mL) was mixed with pepsin solution (pH 1.9) and incubated for 30 min at 37 °C. Following the incubation, pH was altered to 7.5 by using NaOH. Pancreatin solution was then added and solution was incubated for 6 h at 37°C. The mixture was then mixed (1:1) with TCA solution (20 g/100 mL) and centrifuged. The soluble protein in obtained the supernatant was then measured by using Lowry method. BSA was used as standard. Soluble protein was compared to total protein.

Statistical analysis

Data were analyzed by ANOVA and differences between means by Duncan test using SPSS (Ver.22, Chicago, IL). Significance was considered at the level of 5%.

3 Results and discussion

3.1 Proximate composition

Table 1 shows the chemical composition of SPI and water-soluble flour from soy, soy tempe, and germinated-soy tempe. There is no significant difference in moisture content among samples. Meanwhile, ash, protein, and carbohydrate content of water-soluble flour were significantly different ($p < 0.05$) from SPI. Ash as water-soluble component was solved during extraction which then resulted in water-soluble flour with high ash content. The fat content of the SPI was like SF, but both values were significantly lower ($p < 0.05$) compared to those of STF and GTF.

The low protein content of water-soluble flour affected the physical functionality of the flour. Maillard reaction can be initiated by the availability of carbohydrate and protein in alkali condition (Astawan et al., 1994a, b), therefore, high carbohydrate content may result in flour with darker color and lower protein digestibility. Extraction need to be optimized to increase protein content and reduce carbohydrate content of water-soluble flour.

Table 1. Chemical composition of water-soluble flour and soy protein isolate[1].

Sample	Moisture (%)	Ash (%) (dry wt.)	Protein (%) (dry wt.)	Fat (%) (dry wt.)	Carbohydrate(%) (dry wt.)
SF[2]	7 ± 2[ab]	18 ± 0[d]	50 ± 1[b]	0 ± 0[a]	32 ± 1[c]
SPI[3]	5 ± 0[a]	6 ± 0[a]	84 ± 1[c]	0 ± 0[a]	10 ± 1[a]
STF[4]	8 ± 0[ab]	15 ± 0[c]	47 ± 0[a]	8 ± 0[c]	31 ± 0[c]
GTF[5]	9 ± 0[b]	14 ± 0[b]	51 ± 0[b]	7 ± 0[b]	28 ± 0[b]

[1]Different letters indicate significant differences among samples within the same column ($p < 0.05$). Data are means ± standard deviation. [2]Water-soluble flour of Soy. [3]Soy Protein Isolate (commercial). [4]Water-soluble flour of Soy Tempe. [5]Water-soluble flour of Germinated-soy Tempe.

3.2 *Molecular weight of protein*

The electrophoresis patterns are shown in Figure 1. In Figure 1a, the presence of high molecular weight bands is the distinguishing characteristics of non-fermented samples (lane A and B). The fermentation during tempe preparation results in partially hydrolyzed proteins (Bavia et al., 2012). Thus, higher molecular weight polypeptides of STF and GTF (lane C and D) are suggested to be hydrolyzed into lower molecular weight polypeptides as shown in Figure 1b (lane C and D).

3.3 *Trypsin inhibitor activity*

Trypsin inhibitors are antinutrient compounds that interfere with the activity of trypsin. The presence of trypsin inhibitors was shown in Figure 2.

Figure 2 shows lower trypsin activity in SPI, compared to water-soluble flours. Thermal treatment was expected as contributor to the reduction of trypsin inhibitor activity. Radha et al. (2008) reported that thermal process correlated with the reduction of trypsin inhibitor activity.

The high trypsin activity indicated low or no trypsin inhibitor. Several treatments in tempe processing resulted in STF and GTF with no trypsin inhibitor. Egounlety & Aworh (2003) explained that soaking, dehulling and thermal treatment during tempe processing reduced trypsin inhibitors. About 80% of trypsin inhibitor activity from soybean was reduced after tempe processing (Bavia et al. 2012).

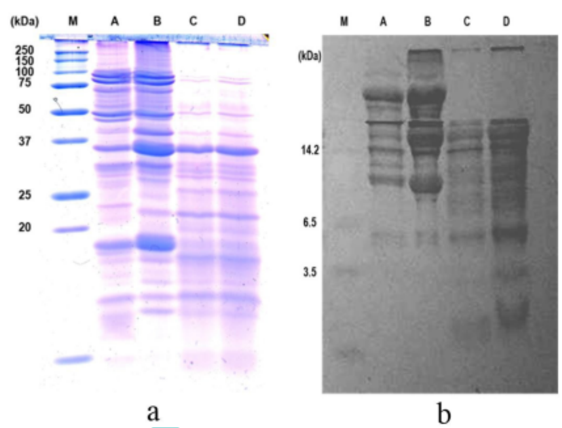

Figure 1. Glycine-SDS-PAGE (a) and Tricine-SDS-PAGE (b) of soy protein isolate (B) and water-soluble flour from soy (A), soy tempe (C), and germinated-soy tempe (D). The lane M is molecular weight markers.

3.4 *Phytic acid*

Phytic acid reduces the bioavailability of protein and minerals. Therefore, the presence of phytic acid in soy-based products is undesirable. Figure 3 provides phytic acid contents of SPI and water-soluble flour. The result showed that phytic acid contents among all samples were significantly different ($p < 0.05$) to each other. The phytic acid content of SF was significantly higher ($p < 0.05$) than others, while that of GTF was significantly lower ($p < 0.05$) than others. A reduction of the phytic acid content during the germination has been reported. Rusydi & Azrina (2012) explained that endogenous phytase activity and the leaching out process during soaking could be the reason of the phytic acid reduction during germination.

The fermentation process also had significant contribution to the reduction of phytic acid contents. Water-soluble flour from fermented soy (STF and GTF) showed significantly lower ($p < 0.05$) phytic acid content to a water-soluble flour from unfermented soy (SF). In accordance with our result, Haron & Raob (2014) reported that tempe processing significantly reduced phytic acid contained in soybean.

3.5 *Antioxidant activity*

Food ingredient with antioxidant activity is suggested to have beneficial effect on health and preservation impact on food system. Figure 4 shows that the DPPH scavenging activities were significantly different ($p < 0.05$) among all groups. The SPI showed the lowest antiradical activity compared to the water-soluble flours. Meanwhile, the STF and GTF had significantly greater ($p < 0.05$) antioxidant activity than the SF. During the fermentation of tempe, bioactive peptides were produced (Gibbs et al., 2004). Moreover, according to Chang et al. (2009), tempe had higher antioxidant activity than unfermented soybeans due to isoflavones-derived compound and hydrolyzed peptides produced during fermentation. Production of hydrolyzed peptides in STF and GTF was also shown in this study as evidenced in the SDS-PAGE profiles (Figure 1).

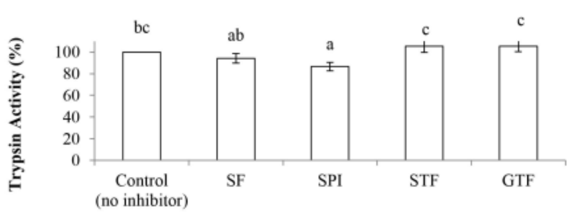

Figure 2. The activity of trypsin with a presence of soy protein isolate (SPI) and water-soluble flour from soy (SF), soy tempe (STF), and germinated-soy tempe (GTF). Different letters indicate significant differences (P < 0.05).

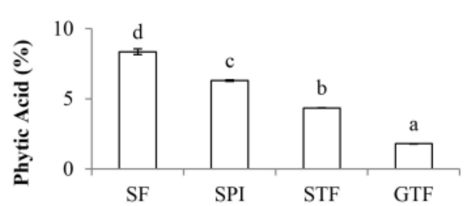

Figure 3. The concentration of phytic acid in soy protein isolate (SPI) and water-soluble flours from soy (SF), soy tempe (STF), and germinated-soy tempe (GTF). Different letters indicate significant differences ($p < 0.05$).

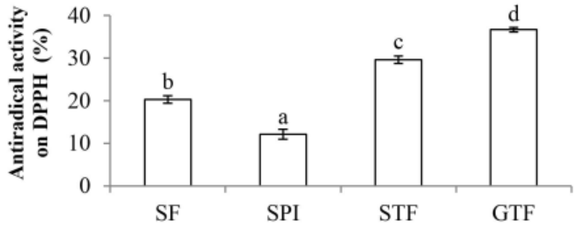

Figure 4. DPPH scavenging activity of soy protein isolate (SPI) and water-soluble flour from soy (SF), soy tempe (STF), and germinated-soy tempe (GTF). Different letters indicate significant differences ($p < 0.05$).

Table 2. Foaming properties of water-soluble flour and soy protein isolate[1].

Sample	Foam Capacity (%)	Foam Stability (%)	Emulsifying Activity Index (m^2/g)	Emulsion Stability Index (min)
SF[2]	26 ± 3^{c}	80 ± 1^{b}	27 ± 1^{c}	103 ± 3^{c}
SPI[3]	14 ± 1^{a}	43 ± 3^{a}	21 ± 0^{a}	30 ± 2^{a}
STF[4]	19 ± 1^{b}	44 ± 2^{a}	24 ± 0^{b}	59 ± 4^{b}
GTF[5]	20 ± 1^{b}	45 ± 2^{a}	24 ± 0^{b}	62 ± 2^{b}

[1]Different letters indicate significant differences among samples within the same column ($p < 0.05$). Data are means ± standard deviation. [2]Water-soluble flour of Soy. [3]Soy Protein Isolate (commercial). [4]Water-soluble flour of Soy Tempe. [5]Water-soluble flour of Germinated-soy Tempe.

Among water-soluble flours, the GTF had the highest antiradical activity. This result agrees with that of Astawan et al. (2016b) who found that tempe flour based on germinated soybeans had greater antioxidant activity than tempe flour based on soybeans due to phenolic compounds and vitamin E improvement. Fernandez-Orozco et al. (2008) also found that germinated soybeans had greater antioxidant activity than non-germinated ones due to bioactive compounds produced during the germination. Germination enhanced the content of ascorbic acid, phenolic compounds, and isoflavones, which were responsible for improving the antioxidant capacity of soybeans (Huang et al. 2014).

3.6 Foaming capacity and stability

The foamability of protein is an ability of the protein to trap gas by forming a thin liquid film. Foamability is becoming important for several food systems such as ice cream, cake, and confectionery products. To provide good foaming properties, proteins must be capable of diffusing in an air-water interface. Foaming properties are usually described by a foaming capacity and a foam stability. The foaming capacity (FC) expresses volume of formed foam after homogenization, while the foam stability (FS) expresses volume of remained foam after a specific time.

The FC and FS of samples are indicated in Table 2, respectively. As expected (Kaur & Singh, 2007; Eltayeb et al., 2011), the protein concentration had a correlation with foam properties. The SPI, which is high in protein, showed significantly higher ($p < 0.05$) FC and FS compared to water-soluble flours.

The fermentation had a positive effect on foam formation. The STF and GTF with hydrolyzed protein performed significantly higher ($p < 0.05$) FC than SF. Molina Ortiz & Wagner (2002) explained that protein with low molecular size was easily migrated and remained in air-water interface, similar with the current results. However, there is no impact of fermentation on the stability of a foam. The FS of STF and GTF were not significantly different with SF. Previous reports showed that the high FC did not always result in high FS, and vice versa. Jitngarmkusol et al. (2008) explained that big bubble with less flexible surface protein could easily collapse. High surface hydrophobicity is needed to allow the formation of stable foam (Molina Ortiz & Wagner, 2002). Moreover, lipid might disturb the stability of the film which is formed by proteins (Kinsella, 1979). Therefore, defatting process is important for the foam properties. These results suggest that the STF and GTF had a chance to be developed as food ingredient for food system that requires foaming properties.

3.7 Activity and stability of emulsion

High activity and stability emulsion is required for water-oil food system. In this study, the emulsion properties were described by the emulsifying activity index (EAI) and emulsion stability index (ESI) in Table 2. The SPI showed significantly higher EAI and ESI compared to water-soluble flours. High concentration of protein supports the reduction of surface tension (Kinsella, 1979).

The STF and GTF showed significantly higher ($p < 0.05$) EAI and ESI than SF. The result revealed that the fermentation during the tempe processing improved emulsion properties. A similar result in fermented peanut flour was reported by Yu et al. (2007). They stated that the protein degradation by proteases improved the solubility of proteins and resulted in the exposure of hydrophobic groups. There is no significant difference between STF and GTF, suggesting no effects of germination of soybeans on the emulsion properties. Overall, the STF and GTF might be developed for water-oil food system such as salad dressing and sausage.

3.8 Protein digestibility (in vitro)

The protein digestibility of water-soluble flours and the SPI is shown in Figure 5. According to Sarwar Gilani et al. (2012), the protein digestibility is correlated with the amount of trypsin inhibitors and phytic acid in the samples and the structure of the proteins. In the current study, trypsin inhibitor may have low impacts on protein digestibility, since trypsin inhibitor activity in SPI and all water-soluble flours are low (Figure 2).

However, current results have proved that phytic acid and the hydrolyzed form of protein influenced the profile of protein digestibility. Sample with higher phytic acid content showed lower protein digestibility. Partial hydrolysis during tempe preparation might provide peptides which were more accessible to digestive enzymes. Based on the SDS-PAGE profile, the STF and GTF contained hydrolyzed peptides with lower molecular weight compared to SF and SPI.

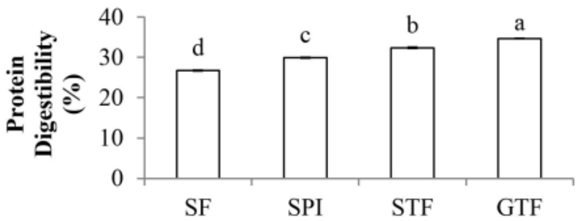

Figure 5. *In vitro* protein digestibility of soy protein isolate (SPI) and water-soluble flours from soy (SF), soy tempe (STF), and germinated-soy tempe (GTF). Different letters indicate significant differences ($p < 0.05$).

4 Conclusion

Overall, the water-soluble tempe flours showed better functional characteristics and nutritional value than the water-soluble soybean flour. Fermentation during tempe processing resulted in water-soluble flours with smaller protein size, higher antioxidant activity, and lower phytic acid content than the soybean based flour. Water-soluble flour had lower functional properties than soy protein isolate due to low protein content. A significant correlation of fermentation to foaming and emulsion properties was observed. The STF and GTF had a potential to use as food ingredient for food system that requires foaming and emulsion properties. However, optimization of the extraction process is needed to improve the protein content.

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