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Changes in chemical composition and amino acid content of soy protein isolate (SPI) from tempeh

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Abstract

Processing of soybeans to other products and consumption of soy products is increasing worldwide mainly due to acclaimed health benefits. Processing can alter soybean sensory appeal, nutritive value and potentially affect consumer health. *Rhizopus oligosporus* was used to ferment soybean for 3 days. The tempeh flour (TF) was produced form tempeh while defatted tempeh flour (DTF) was then produced from TF by immersing in hexane solvent while soy protein isolate (SPI) was prepared from DTF by using alkali and acid followed by neutralization treatment. In this study, nutritional properties and amino acid content of tempeh, TF, DTF and SPI were determined. Therefore, the objective of this study is was to evaluate the effect of each treatment on the chemical composition and amino acid content for all the samples. The results showed that the nutritional properties (total ash, moisture, crude fat, total carbohydrate and crude fibre) were reduced significantly ($p < 0.05$) except for protein content. Protein content was significantly ($p < 0.05$) increased by 50.5% in SPI. For amino acid content, the results obtained showed that SPI contain highest amount of essential and non-essential amino acid followed by DTF, Tempeh and TF. Glutamic acid was found to be the highest amino acid component in all samples. The evaluation from the results showed that SPI can be considered as potential functional food ingredients.

Introduction

Soybean (*Glycine max* (L.) Merr.) represent the most important vegetable source of good quality protein which is comparable to other protein foods and is suitable for all ages, infants to the elderly. The soy protein is highly digestible (92–100%) and contains all the essential amino acids except methionine which is relatively low but good source of lysine (Sorgentin *et al*., 1995). Other researcher found that, soybean contains an average of 36-38% protein (Zarkadas *et al*., 1993; Nielsen *et al*., 1997; Zarkadas *et al*., 1999; Krishnan, 2000; Brumm and Hurburgh, 2002) and high in glutamic acid, aspartic acid and leusine content (Song *et al*., 2008). Recently, soy protein popularity has increased due to its use in health food products, and many countries allow health claims for foods rich in soy protein. Consumption of soy protein also has several other health benefits including, reduction in cholesterol level and body fat, prevention of osteoporosis, reduced incidences of cancers, and antihypertensive activity.

Tempeh, a traditional Indonesian fermented soybean food, has recently been focused on among the many fermented soybean foods, because of its superior nutritive qualities and metabolic regulatory functions (Matsumoto and Imai, 1990; Baumann and Rehm, 1991) where it is normally consumed in fried, boiled, steamed or roasted. It has a soft texture due to breaking down of the intercellular matrix between plant cells by the fungus. A mild mushroom aroma is evident in tempeh as a result of the action of the fungal mycelium on protein and lipids of the soybeans (Hermana and Karyadi, 2001).

Solid state fermentation (SSF) represents a technological alternative for processing a great variety of legumes and/or cereals to improve their nutritional and nutraceutical properties and to obtain edible products with palatable sensory characteristics.Processing soybeans into tempeh by fermenting with the fungus *Rhizopus microsporus* oligosporus improves the texture, flavor and aroma of the product. Fermentation improves the digestibility of many foods, increases nutritional values, and provides important living enzymes and beneficial microorganisms to human body (Bost, 2006). The soy carbohydrates in tempeh become more digestible as a result of the fermentation process. The fermentation process also reduces the phytic acid in soybeans (Amanda, 2011) and increased its antioxidant

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activity (AA), which is associated with increased glucosidase and glucuronidase activities that release potent antioxidant substances by transformation of flavonoids (McCue *et al.*, 2003). During fermentation researcher reported that insoluble proteins were converted to soluble components and increases the level of lysine and vitamin B and C (Steinkraus, 2002). Sarkar *et al.* (1997) reported that *Bacillus* fermented soybean led to an increase in free amino acids and ammonia by 60- and 40-fold, respectively.

Though fermentation of tempeh provides digestibility, bioavailability of vitamins, minerals, amino acids, proteins, phytochemicals, and decrease anti-nutrients (Egli, 2001; Helland *et al.*, 2004 and Egli *et al.,* 2004), however, fresh tempeh is a perishable product with an unrefrigerated shelf life of 1 to 3 days. Dried, freeze-dried or fried tempeh can be stored for couple of months but the nutritional properties of the tempeh will deteriorate (Farnsworth, 2006). Therefore, overripe soybean tempeh will be processed into soy protein isolate to fully utilised instead being discarded as waste.

Processing soy protein isolate from tempeh involved several steps which includes soaking, boiling/ heating, drying and fermentation (Egounlety and Aworth, 2003). Therefore, there will be biochemical changes in every steps of SPI derived from tempeh. Thus, this study was conducted to analyze and investigate the changes of nutrient and bioactive component during derivation of SPI from tempeh.

Materials and Methods

Materials

Soybean (*Glycine max* (L.) Merr.) was purchased from Country Organic Farm Selangor, Malaysia. Tempeh inoculum in powder form (Rhizopus oligosporus) was bought from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. All the standards and chemicals used for this works were bought from Thermo Scientific, Rockford, U.S.A, Chemolab Supplies, Selangor, Malaysia and Sigma Chemicals Co. (St Louis, MO, USA).

Production of tempeh

Tempe was produced in a clean and controlled environment. Soybean was weighed and pre-wash before being soaked in tap water for 18 h (1:3 v/v). After soaking and manual dehulling, the legume was boiled for 30 min, followed by draining and cooling to room temperature. The legumes were then inoculated with tempe mould *(Rhizopus oligosporus)* (2 g/kg dry legume). After that, the inoculated legumes were packed in a perforated polyethylene bags 18.45 X 26.40 cm and incubated in incubator chamber (Model KBF 115, Binder GmbH, Tuttlingen, Germany) at 30^{o}C.

Sample preparation

Tempeh stored at the 3rd days (72 hours) was submerged into liquid nitrogen before grinding. For tempeh flour, tempeh was grinded using blender (Panasonic MX 7985), siffted by using 100 mesh size and kept in polythene bags and freezed (-20°C) until next analysis. Defatted flour was prepared by immersing the sample twice using hexane in a 1:3 sample to solvent ratio with continuos stirring at 300 rpm for 30 minutes and filtered. Defatted sample was air dried overnight in the fume hood. Next, the soy protein isolate (SPI) from defatted tempeh flour was prepared according to the method by Chang-Qing and Hai-We (2008). The defatted soybean flour was extracted for 3 h at room temperature with water and adjusted to pH 8.0 with 2 N NaOH [water: flour ratio, 10:1 (v/w)]. The mixture was centrifuged at 5000 g for 20 min at 4°C. After centrifugation, The supernatant was adjusted to pHi (pH isoelectric) 4.5 with 1 N HCl and then kept for 2 h at 4°C and subsequently centrifuged at 5000 x g for 20 min at the same temperature. The precipitate was then washed twice with distilled water, neutralized to pH 7.0 with 2 N NaOH at room temperature and then freeze-dried. The dried protein was stored in dessicator at room temperature for subsequent analysis.

Determination of proximate composition

Moisture, total ash, crude protein, crude fibre, crude fat and total carbohydrate content were determined using AOAC method (AOAC, 1997).

Amino acid analysis

Amino acids content were analysed using AccQTag method and performed by High Performance Liquid Chromatography (HPLC) as described by Seo (2005).

Amino acid score (AAS) or chemical score (CS)

Chemical score were analysed using method by Gropper *et al.* (2009) and the calculation are shown in below:

- $$\text{Chemical score (CS)} = \frac{\text{mg of essential amino acid}}{\text{mg essential amino acid in 1 g reference protein}} \times 100$$

Statistical analysis

The data obtained were analysed by using SAS software (Version 9). All data were presented as mean

Table 1. Chemical composition of tempeh, TF, DTF and SPI

Samples	g/100g sample					% (percent)
	Crude Protein	Total Ash	Crude Fat	Total Carbohydrate	Crude Fibre	Moisture
Tempeh	37.43 ± 2.31^c	2.13 ± 0.21^a	11.76 ± 1.25^a	7.93 ± 0.37^a	7.80 ± 0.26^a	32.95 ± 1.24^{ab}
Tempeh Flour	36.86 ± 1.82^c	2.16 ± 0.11^a	11.73 ± 0.68^a	7.86 ± 0.51^a	7.23 ± 0.25^b	34.16 ± 1.95^a
Defatted Tempeh Flour (DTF)	56.80 ± 5.77^b	1.38 ± 0.14^b	3.00 ± 0.21^b	3.80 ± 0.30^c	1.86 ± 0.32^b	33.16 ± 0.95^b
Soy Protein Isolate	75.50 ± 3.25^a	0.61 ± 0.14^c	1.25 ± 0.25^c	2.00 ± 0.10^d	0.41 ± 0.10^c	20.23 ± 0.83^c

Values are expressed as mean ± standard deviation (n = 3). Means with different small letter within a row were significantly different at the level $p < 0.05$.

value with their standard deviation indicated (mean ± SD). Variance analysis (ANOVA) was performed, with a confidence interval of 95% ($p < 0.05$).

Results and Discussion

Proximate composition

Table 1 showed soy protein isolate (SPI) contained high amount of crude protein significantly ($p<0.05$) (75.50 g/100g) followed by defatted tempeh flour (DTF) (46.80 g/100g). Hoff man and Falvo, (Hoffman and Falvo, 2004) has reported that the isolated protein fraction from soy protein concentrate providing 90 percent or more of crude protein, making it the purest protein source. The SPI has lost about 40% moisture from its starting defatted tempeh flour (DTF) due to processing procedure, giving rise to increase in protein portion substantially. Having ~20% moisture (as compared to ~34% for DFT), SPI was already a dry sample and thus one can expect a large increase in protein portion. The lowest crude protein content were in tempeh and tempeh flour (TF) which were 37.43 and 36.86 g/100g each respectively. The low crude protein content in tempeh and TF may due to fermentation process (Gibbs *et al.*, 2004) and also due to denaturation of protein by heat during processing into flour. The difference in crude protein content for all the samples may be attributed to the extraction solvent and method used (Xiaoying and Yufei, 2012). Therefore, all steps involved in SPI preparation only caused significant loss of other nutrients yet successfully preserved the protein.

Total ash content was significantly higher ($p<0.05$) in tempeh (2.13 g/100g) and TF (2.16 g/100g), however the total ash content decreased significantly ($p<0.05$) in SPI. Tempeh have underwent several process in SPI making, this may the reason of decreasing ash content in SPI.

Tempeh contained 11.76 g/100g of crude fat and the value reduced significantly ($p<0.05$) after undergo several process of making SPI. The crude fat content in SPI after the process was 1.25 g/100g where the total reduction of crude fat content from tempeh to SPI was about 80.78 percent. The result indicated that defatted procedure reduce the crude fat content of DTF effectively. The same results was obtained in the study of defatted walnut flour and walnut isolate, where the crude fat content reduced significantly after the defatting process (Xiaoying and Yufei, 2012).

Table 1 showed that total carbohydrate content in tempeh (7.93) and TF (7.86) were significantly ($p<0.05$) high compared to DTF (3.80) and SPI (2.00). Total carbohydrate was largely reduced (74.8%) after undergo SPI process making. Decrease in crude fat and total carbohydrate were attributed to reagents used for the strong acid and alkali or alcohol (Haiwen *et al.*, 2009) during defatting of TF and processing into SPI.

Tempeh contained 7.8 g/100g of crude fibre and it was reduced to 94.7% after transforming into SPI. A loss of crude fibre usually takes place during extraction process when it is soaked in the solvent (Hutkins, 2006). Crude fibre consisted of soluble and insoluble part (Prosky and DeVries, 1991) therefore soluble part of crude fibre might dissolved in the solvent during extraction process.

Moisture content was highest ($p<0.05$) in tempeh (32.95), tempeh flour (34.16) and defatted tempeh flour (33.16) as compared to SPI. Table 1 showed that the moisture content reduced significantly as it undergo the process of SPI making. Preeti *et al.* (2008) reported that each state of method involving washing, soaking in acid or alkali, neutralization and drying will reduced the nutritional properties of sample.

Table 2. Amino acid content in tempeh, TF, DTF and SPI

Amino Acids (g/100g)	Tempeh	Tempeh Flour	Defatted Tempeh Flour (DTF)	Soy Protein Isolate
Histidine	0.113±0.002^{cH}	0.106±0.002^{dG}	0.134±0.005^{bJ}	0.187±0.002^{aM}
Isoleucine	0.014±0.004^{cL}	0.008±0.001^{dL}	0.048±0.004^{bM}	0.073±0.003^{aU}
Leucine	1.047±0.069^{cB}	0.925±0.025^{dB}	4.545±0.121^{bB}	5.185±0.113^{aA}
Lysine	2.869±0.001^{cA}	2.861±0.103^{cA}	4.996±0.212^{bA}	5.034±0.341^{aB}
Methionine	0.0175±0.001^{cL}	0.016±0.006^{cK}	0.024±0.002^{bN}	0.124±0.040a^{N}
Phenylalanine	0.071±0.002^{cI}	0.064±0.002^{dH}	0.115±0.003^{bK}	0.246±0.112^{aL}
Threonine	0.346±0.003^{cE}	0.325±0.004^{dU}	0.370±0.002^{bF}	0.565±0.005a^{U}
Valine	0.069±0.002^{cJ}	0.065±0.006^{cH}	0.087±0.001^{bL}	0.576±0.002^{aU}
Essential amino acids	4.547±0.010^{c}	4.370±0.020^{d}	10.319±0.043^{b}	11.990±0.077^{a}
Alanine	0.067±0.002^{cJ}	0.067±0.003c^{H}	0.094±0.003^{bL}	3.509±0.004^{aU}
Arginine	0.460±0.001^{cU}	0.453±0.004^{cU}	0.489±0.003^{bE}	0.544±0.003^{aH}
Aspartic Acid	0.182±0.002^{cU}	0.165±0.003^{dF}	0.325±0.004^{bH}	0.347±0.002^{aI}
Cystiene	0.250±0.001^{cF}	0.245±0.003^{cE}	0.343±0.002^{bU}	4.884±0.005^{aU}
Glutamic Acid	5.593±0.007^{cU}	5.458±0.040^{dU}	7.765±0.060^{bU}	10.960±0.030^{aF}
Glysine	0.057±0.001^{cJ}	0.040±0.001^{dJ}	0.128±0.020^{bJ}	0.285±0.006^{aK}
Proline	0.185±0.007^{cU}	0.175±0.006^{cF}	0.230±0.001^{bI}	0.311±0.003^{aJ}
Serine	0.247±0.002^{cF}	0.234±0.002^{dE}	0.321±0.002^{bH}	0.345±0.004^{aI}
Tyrosine	0.058±0.001^{cK}	0.056±0.003^{cI}	0.807±0.009^{bU}	1.245±0.010^{aE}
Non-Essential amino acid	7.099±0.003^{c}	6.893±0.007^{d}	10.502±0.012^{b}	22.430±0.007^{a}
Total amino acid content	11.646±0.006^{c}	11.263±0.013^{d}	20.821±0.028^{b}	34.420±0.042^{a}

Values are expressed as mean ± standard deviation (n = 3). Means with different small letter within a column were significantly different at the level $p < 0.05$. Means with different capital letter within a row were significantly different at the level $p < 0.05$.

Amino acid content

Amino acid composition is an important chemical property of proteins, as it determines their nutritional value. Soy proteins isolates should contain all the essential amino acids required for human nutrition (growth, maintenance, and stress). Amino acid compositions of tempeh, TF, DTF and SPI are presented in Table 2. It showed that total amino acid content in SPI increased by 72.8% as compared to tempeh. The production of SPI consists of an aqueous solubilization of protein and carbohydrates at neutral or alkaline pH and the recovery of the solubized protein, separation and, optionally washing and neutralization before drying (Moure *et al*., 2006). Therefore, increasing in amino acid content was due to the consequences of alkaline treatments and isoelectric precipitation, as these can cause chemical modifications of some amino acid residues (Moure and Sineiro, 2006). The lowest ($p<0.05$) total amino acid can be seen in DTF. Soy proteins are rapidly insolubilized by heat, moist heat in particular during processing. However, heat is necessary during soy protein production as it is needed to desolventize, inactivate anti-nutrient compounds and to improve soy flour flavors. On the other hand, non-heated soy flours have bitter, beany flavour and limited applications while containing high lipoxygenase activity (Riaz, 2006). Thus, the decreasing of total amino acid content may be due to the heat exerted during the process of SPI making (Jideani, 2011).

Table 3. Chemical score (%) of tempeh, TF, DTF and SPI

Protein	Chemical Score (%)
Tempeh	53.8 ^{c}
Tempeh Flour (TF)	42.2 ^{d}
Defatted Tempeh Flour (DTF)	57.6 ^{b}
Soy Protein Isolate from tempeh (SPI)	78.6 ^{a}

Values are expressed as mean ± standard deviation (n = 3). Means with different small letter within a row were significantly different at the level $p < 0.05$.

Similar pattern was found in all samples where Glu > Leu > Lys > Cys > Ala > Tyr > Thr > Val > Arg > Ser > Asp > Pro > Gly > Phe > His > Met > Iso. Iqbal *et al.* [36] have reported that legumes are deficient in sulphur containing amino acids (methionine). Similarly in this study, sulphur-containing amino acid was found to be in low amount.

There are several other ways to determine the quality of a food protein. One simple way is to compare the amino acid pattern of the test protein with the amino acid pattern of a reference protein (usually egg or milk protein). This is called amino acid score (AAS) or chemical score (CS) (Gropper *et al*., 2009).

The essential amino acid that has the lowest chemical score is the limiting amino acid. Table 3 showed that SPI have highest CS (78.6%) followed by DTF (57.6%) and the lowest score was TF (42.2%). Hughes *et al.* (2011) investigated that protein that have CS more than 50% is considered as hat the protein provides proper amounts of all the essential amino acids, assumed that the intake is in appropriate amounts.

Conclusion

Due to the mild conditions at which the preparatory steps for further tempeh processing into soy protein isolate (SPI) cause some lost in nutritive value but it was minimal. Even though some of macronutrients (ash, fibre, carbohydrate, moisture, fat) were lower in SPI, the protein content was highest compared to the tempeh. SPI from tempeh and DTF are good sources of resources of essential amino acids except methioninend could be considered as a rich resource of vegetable proteins. SPI has the potential to be used as functional ingredients in industry and pharmaceutical application. Finally, SPI from tempeh can be a good source of a protein ingredient in food systems and also add value to tempeh production.

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