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Artikel Asli/*Original Article*

Nutrient Contents in *Tempe* Produced from Five Cottage Industries in Selangor, Malaysia

(Kandungan Nutrien dalam Tempe yang Dihasilkan dari Lima Industri Desa di Selangor, Malaysia)

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ABSTRACT

This study aimed to determine the nutrient contents in tempe produced by five cottage industries in Selangor, Malaysia. Proximate contents were analysed by using standard methods of AOAC (1997) while carbohydrate content was calculated by difference. Mineral contents, total dietary fiber (TDF), total phenolic content and total isoflavone content were determined by Atomic Absorption Spectrophotometry (AAS), enzymatic-gravimetric (AOAC 985.29), Folin-Ciocalteu colorimetric and High Performance Liquid Chromatography (HPLC) respectively. Macronutrients were reported in 100 g sample and the results showed the average nutrient contents were as follow: 63.07 ± 3.18% moisture, 19.63 ± 1.50% protein, 0.65 ± 0.17% fat, 0.70 ± 0.06% ash and 15.95 ± 1.88% total carbohydrate. The average mineral content in 100 g samples (based on wet basis) were 29.45 ± 5.67 mg calcium, 13.28 ± 5.76 mg magnesium, 3.48 ± 1.09 mg sodium and 2.06 ± 0.33 mg ferum. The results showed that the average of TDF content was 8.05 ± 3.65%. Total phenolic content was 259.87 ± 22.62 mg of GAE/g. The total isoflavone content in 100 g samples (wet basis) was 41.94 ± 10.42 mg/100 g. This study had shown that total phenolic content was significantly correlated ($p < 0.01$) with total isoflavone content in all tempe samples. It can be concluded that there was no significant difference ($p > 0.05$) in nutrient contents among tempe samples produced by five cottage industries located in Selangor, Malaysia. However, the mineral and isoflavone contents in the present study were lower compared to previous studies.

Keywords: Raw tempe; total phenolic content; isoflavone, macronutrient; small scale industries

ABSTRAK

Kajian ini dijalankan untuk menentukan kandungan nutrien dalam tempe yang dihasilkan di lima industri desa yang terletak di Selangor, Malaysia. Kandungan proksimat dianalisis dengan menggunakan AOAC (1997) manakala jumlah kandungan karbohidrat ditentukan melalui kaedah perbezaan. Kandungan mineral, serat diet total (TDF), jumlah fenolik dan kandungan isoflavon, masing-masing ditentukan melalui kaedah spektrofotometer serapan atom (AAS), enzimatik-gravimetrik (AOAC 985.29), kaedah Kolorimetrik Folin-Ciocalteu dan kromatografi cecair berprestasi tinggi (HPLC). Purata kandungan makronutrien yang dikaji masing-masing adalah 63.07 ± 3.18% bagi kandungan air, 19.63 ± 1.50% bagi kandungan protein, 0.65 ± 0.17% bagi kandungan lemak, 0.70 ± 0.06% bagi kandungan abu dan 15.95 ± 1.88% bagi jumlah kandungan karbohidrat. Purata kandungan mineral dalam 100 g sampel adalah 29.45 ± 5.67 mg kalsium, 13.28 ± 5.76 mg magnesium, 3.48 ± 1.09 mg natrium serta 2.06 ± 0.33 mg ferum. Kajian ini mendapati tempe yang dikaji mengandungi purata kandungan serat diet total sebanyak 8.05 ± 3.65%. Hasil kajian ini mendapati tiada perbezaan signifikan ($p > 0.05$) bagi kandungan proksimat, mineral dan serat diet total dalam semua sampel. Purata jumlah kandungan fenolik adalah 259.87 ± 22.62 mg of GAE/g manakala purata kandungan jumlah isoflavon didapati sebanyak 41.94 ± 10.42 mg/100 g berat basah. Hasil kajian ini menunjukkan bahawa jumlah kandungan fenolik berkorelasi secara signifikan ($p < 0.01$) dengan kandungan jumlah isoflavon. Secara keseluruhan, tiada perbezaan yang signifikan dalam nilai kandungan nutrien antara tempe-tempe yang dihasilkan di industri desa di Selangor, Malaysia. Namun, kandungan mineral dan kandungan isoflavon (Da) kajian ini didapati lebih rendah berbanding dengan kajian-kajian lepas.

Kata kunci: Tempe mentah; jumlah kandungan fenolik; isoflavon; makronutrien; industri desa

INTRODUCTION

Small scale industry is essential in contributing to the economic development and it can be established for any kind of business activities in urban or rural area. It can be considered as the backbone of the national economy (Bramsiepe et al. 2012). This small scale industry will ensure the food security for the increasing population in urban area (Rolle & Satin 2002; Bramsiepe et al. 2012).

Majority of fermented food are produced using traditional methods at both cottage and small-scale industries of developing countries (Rolle & Satin 2002;

Valyasevi & Rolle 2000). Fermented food represents one-third of total food consumption and one of it is *tempe*, a major fermented soybean food (Nouts & Kiers 2005). Fermentation process of *tempe* increases the nutritional values of some nutrients, development of vitamins, phytochemicals and antioxidative constituents (Astuti & Dalais 2000). However, there is no standard process for *tempe* making and that is why there are many variations in *tempe* making at different region or by different producer (Astuti et al. 2000). *Tempe* is normally produced by cottage industry in Malaysia (Hasnah et al. 2009). However, database for nutritional value of *tempe* from local production is not available. Thus, this study was initiated to investigate the nutritional values of *tempe* produced by five *tempe* producers in Selangor.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of five different cottage industries of *tempe* production located in Selangor were selected. Raw *tempe* were purchased from each of the industries, which were Taman Enquine, Taman Universiti Indah, Puchong, Klang Lama and Selayang. Convenience sampling was used to obtain the samples. Sampling was carried out twice at two different times. A total of four replicates were analysed for each sample.

MACRONUTRIENT CONTENT

Tempe samples were ground into fine particles and analyzed for moisture, crude protein, crude fat and ash (in wet basis) according to AOAC method (1997). Total carbohydrate content was calculated by difference. Enzymatic-gravimetric method (Prosky et al. 1985) was used to determine the total dietary fiber (TDF) content.

MINERAL CONTENT

Mineral content such as calcium (Ca), magnesium (Mg), sodium (Na) and Ferum (Fe) were determined using Atomic Absorption Spectrophotometer (AAS). Standard stock solution of Ca, Mg, Na and Fe were prepared from AAS grade chemicals (Fisher scientific, UK) with appropriate dilutions.

TOTAL PHENOLIC CONTENT (TPC)

The amount of total phenolics in the samples was determined using the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE) (Akitha Devi et al. 2009). The extracted sampel (50 µl), distilled water (3 µl), Folin-Ciocalteu reagent (250 µl) and 7% sodium carbonate (750 µl) were mixed together and incubated for 8 minutes at room temperature. About 950 µl distilled water added into the mixture and the mixture was left at room temperature for 2 hours. The absorption at 765 nm was performed using UV-visible spectrophotometer and distilled water was used as blank.

DETERMINATION OF TOTAL ISOFLAVONE CONTENT (DAIDZEIN AND GENESTEIN)

All samples were freeze-dried and ground into fine particles before analyzed. The freeze-dried samples were kept in containers and stored at -20°C until further analysis. The extraction of isoflavone was performed as reported previously (Hutabarat et al. 2000). The finely ground sample (1 g) was added to 10 ml of 2 M HCl and 40 ml of 96% ethanol (containing 60 ppm of flavones). The samples mixture was then placed in sonicator for 20 min before heated in water bath at 100°C and refluxed for 4 hours. The samples mixture was made up to 50 ml and adjusted to pH 4 with sodium hydroxide followed by centrifugation for 20 min. The clear supernatant was injected into a reverse phase of high performance liquid chromatography (HPLC) after filtered through 0.20 µm polytetrafluoroethylene microfilter.

STATISTICAL ANALYSIS

Data were expressed in mean ± standard deviation of four replicate measurements for all the nutrient content analysis except total dietary fiber composition was measured in duplicate measurements. All the laboratory data were analyzed using statistical software, SPSS version 19.0 for windows. One-way ANOVA with Turkey's HSD was used to determine the differences for all nutrients in all samples. Level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION

All the macronutrients in *tempe* samples were shown in Table 1. The average moisture content was 63.07 ± 3.18% and the moisture content measured in *tempe* TE (65.55 ± 0.66%) was significantly higher ($p < 0.05$) compared to *tempe* KLL (63.04 ± 1.11%) and *tempe* S (57.75 ± 0.84%). The average protein content was 19.63 ± 1.50% based on wet weight and it showed no significant difference compared to USDA database (2016) however it was observed to be slightly higher compared to Malaysian Food Composition Database Tee et al. (1997) which contained 20.29% and 15.90% respectively. The average of fat content was 0.65 ± 0.17% based on wet basis and it was relatively much lower compared to the one reported in Tee et al. (1997) and USDA database (2016) which were 7.5% and 10.80%, respectively. Low fat recoveries can be a result of incomplete drying of samples which acts as physical barrier preventing dissolution of the fat into the solvent (Anderson 2004). Ash content was significantly higher ($p < 0.05$) in *tempe* KKL (0.82 ± 0.01) compared to *tempe* TE (0.56 ± 0.03) and *tempe* TUI (0.65 ± 0.03). Among all the samples, the average of ash content was 0.70 ± 0.06% which was lower compared to Tee et al. (1997) and USDA database which reported *tempe* to contain 0.9% and 1.62% ash, respectively. The total carbohydrate content was calculated by difference and the highest carbohydrate content in *tempe* S was 19.06 ± 1.69%, significantly higher ($p < 0.05$) compared to *tempe* KLL (14.44 ± 2.11%), *tempe* TUI (14.81 ± 1.61%) and *tempe* TE (15.08 ± 0.90%). The proximate contents in *tempe* were compared to Malaysian Food Composition Database (FCD) and USDA database. The average of moisture, protein, ash and carbohydrate content (moisture: 63.07%; protein: 19.63%; ash: 0.70%; carbohydrate: 15.95%) were relatively comparable with nutrient contents listed in Tee et al. (1997) and USDA database.

MINERAL CONTENTS

Mineral contents in this present study were showed in Table 2. Calcium (Ca), magnesium (Mg), sodium (Na) and iron (Fe) contents in these studied samples were measured based on wet weight. However, most of the mineral content (Ca: 29.45 ± 5.67 mg/100 g; Mg: 13.28 ± 5.76 mg/100 g; Na: 3.48 ± 1.09 mg/100 g) were reported to be lower compared to Tee et al. (1997) and USDA database (Ca: 69-111 mg/100 g; Mg: 81 mg/100 g; Na: 7-9 mg/100 g). The low ash and mineral content of these samples were consistent as Ogu & Ugwu (2011) stated that ash and mineral contents are interrelated to each other in the food samples. Only the value of ferum content was found similar (2.06 ± 0.33 mg/100 g) to the database of USDA database (2.7 mg/100 g) but slightly higher compared to Malaysian FCD (1.8 mg/100 g).

TOTAL DIETARY FIBER

Table 1 showed there were no significant difference ($p < 0.05$) in total dietary fiber (TDF) content in all *tempe* samples based on wet weight. The average TDF content for all *tempe* samples was 8.05 ± 3.65%. The TDF content in tempe of this study was lower compared to the one (5.6%) in *tempe* reported by Dutch Food Composition Table (2013). However, *tempe* S contained highest amount of TDF (10.58 ± 0.86%) while the TDF content in *tempe* TUI (5.78 ± 1.88%) contained lowest content of TDF when compared to other samples. The difference of total dietary fiber content may be due to different types of soybeans and duration of processing used (Azizah & Zainon 1997; Kutos et al. 2003). TDF value was not included in both Tee et al. (1997) and USDA database. Malaysia FCD reported that *tempe* contained 2.9% of crude fiber. According to Zeman (1991), TDF can be estimated as an approximation of two to six times of crude fiber content. Therefore, the TDF content in *tempe* of this present study was in the range with the crude fiber in Tee et al. (1997), with TDF estimated to be in the range of 5.8-17.4% and the one (9.58%) reported by Hasnah & Norfasihah (2014).

TOTAL PHENOLIC CONTENT

The total phenolic content of this study was expressed in mg of GAE/g unit, as shown in Table 3. The calibration curve for total phenolic content showed linearity of coefficient of determination, $r^2 = 0.998$ by using Gallic acid as standard with concentration ranging from 0 – 250 mg/ml GAE. The average of total phenolic content was 259.87 ± 22.62 mg of GAE/g. *Tempe* TE (284.27 ± 22.47 mg of GAE/g) contained significantly higher ($p < 0.05$) total phenolic content than *tempe* P (233.64 ± 14.56 mg of GAE/g). The existence of reducing agent may be the factor of reducing Folin-Ciocalteu and this may affect the accuracy of the total phenolic content obtained (Tyug et al. 2010).

ISOFLAVONE CONTENT

Total isoflavone content (Daidzein and Genestein) was determined using HPLC method as this method was able to provide optimum resolution, precision and redundancy (Hutabarat et al. 2000). The calibration curve for Daidzein (Da), Genestein (Ge) and Flavone (Fl) showed linearity of coefficient of determination, $r^2 > 0.99$ with concentration ranging from 5-30 μM. Total isoflavone content of *tempe* samples in this study was expressed in the unit mg/100 g based on wet weight.

Table 3 showed the total isoflavone content among all the studied samples. The average isoflavone content in all *tempe* of this study were 2.42 ± 0.39 mg Da/100 g, 42.31 ± 10.68 mg Ge/100 g and 41.94 ± 10.42 mg total isoflavone /100 g. *Tempe* S contained 56.55 ± 12.23 mg total isoflavone/100 g which was significantly higher ($p < 0.05$) compared to *tempe* KLL (35.65 ± 6.16 mg total isoflavone /100 g) and *tempe* P (28.93 ± 0.35 mg total isoflavone/100 g). *Tempe* KLL, TUI and P samples contained 2.41 ± 0.61 mg Da/100 g, 2.35 ± 0.13 mg Da/100 g and 2.33 ± 0.07 mg Da/100 g respectively. GE content in most samples was higher than the one reported by Hasnah et al. (2009) and USDA Database.

The present result was in contrast with previous studies as Da is more stable compared to Ge in temperature between -80°C and 4°C (Eisan et al. 2003; Rostagno et al. 2005). However, soybean is the main ingredient in *tempe* production and isoflavone content could affected by genetic, planting year and planting location of different soybean cultivar (Carro-Panizzi et al. 2009).

In this study, there was a significant ($p < 0.01$) strong positive relationship between total phenolic and total isoflavone contents,with Pearson'rho value, r = 0.704. This indicates that all *tempe* samples in this study that contained high total phenolic content contained high total isoflavone content as well. This result showed relatively good agreement with previous studies which indicated that total phenolic content correlated with total isoflavone content and antioxidant activity in soybean (Devi et al. 2009; Mujic et al. 2011).

TABLE 1. Proximate and total dietary fiber contents in *tempe* samples

Sample	Nutrients (% composition)											
	Moisture	CV	Crude protein	CV	Crude fat	CV	Carbohydrate	CV	Ash	CV	Total Dietary Fiber	CV
Taman Enquine (TE)	65.55 ± 0.66^{a}	1.00	18.50 ± 0.55^{a}	2.97	0.31 ± 0.03^{c}	9.84	15.08 ± 0.90^{b}	5.97	0.56 ± 0.03^{c}	4.72	7.21 ± 3.58^{a}	49.67
Taman Universiti Indah (TUI)	65.45 ± 0.81^{a}	1.24	18.70 ± 0.95^{a}	5.08	0.39 ± 0.18^{bc}	45.58	14.81 ± 1.61^{b}	10.87	0.65 ± 0.03^{bc}	5.18	5.79 ± 1.88^{a}	32.58
Puchong (P)	63.57 ± 1.73^{ab}	2.71	18.41 ± 0.31^{a}	1.69	0.89 ± 0.25^{ab}	28.47	16.34 ± 1.42^{ab}	8.67	0.79 ± 0.15^{ab}	19.69	8.54 ± 1.82^{a}	21.30
Klang Lama (KLL)	63.04 ± 1.11^{b}	1.75	21.27 ± 3.17^{a}	14.88	0.44 ± 0.08^{bc}	17.95	14.44 ± 2.11^{b}	14.63	0.82 ± 0.01^{a}	1.17	8.13 ± 2.84^{a}	34.92
Selayang (S)	57.75 ± 0.84^{c}	1.46	21.28 ± 0.44^{a}	2.07	1.24 ± 0.45^{a}	36.47	19.06 ± 1.69^{a}	8.88	0.67 ± 0.04^{abc}	6.40	10.58 ± 0.86^{a}	8.16
Average	63.07 ± 3.18	5.04	19.63 ± 1.50	7.66	0.65 ± 0.17	25.56	15.95 ± 1.88	11.78	0.70 ± 0.06	8.32	8.05 ± 3.65	45.30

Values for macronutrient contents were averaged from four sample replicates and expressed as mean ± standard deviation.
Values with different alphabets were ~~not~~ significantly different between the samples ($p < 0.05$)

TABLE 2. Mineral content in *tempe* samples

Sample	Mineral content (mg/100 g wet weight)							
	Calcium	CV	Magnesium	CV	Sodium	CV	Iron	CV
Taman Enquine (TE)	27.69 ± 5.29^{bc}	19.09	10.25 ± 0.74^{ab}	7.18	4.05 ± 1.83^{a}	45.28	2.38 ± 2.23^{a}	93.50
Taman Universiti Indah (TUI)	30.58 ± 5.46^{ab}	17.86	8.50 ± 0.35^{b}	4.16	4.09 ± 1.94^{a}	47.32	1.86 ± 0.62^{a}	33.60
Puchong (P)	37.14 ± 1.46^{a}	3.93	22.69 ± 12.63^{a}	55.69	2.22 ± 0.19^{a}	8.37	2.17 ± 0.61^{a}	28.08
Klang Lama (KLL)	30.39 ± 1.65^{ab}	5.44	14.81 ± 1.21^{ab}	8.20	2.41 ± 0.22^{a}	9.20	1.59 ± 0.37^{a}	23.48
Selayang (S)	21.43 ± 2.24^{c}	10.45	10.13 ± 0.97^{b}	9.56	4.62 ± 1.24^{a}	26.78	2.32 ± 0.83^{a}	35.85
Average	29.45 ± 5.67	19.26	13.28 ± 5.76	43.41	3.48 ± 1.09	31.33	2.06 ± 0.33	16.16

Values for mineral contents were averaged from four sample replicates and expressed as mean ± standard deviation.
Values with the different alphabets were ~~not~~ significantly different between the samples ($p < 0.05$)

TABLE 3. Total phenolic content and total isoflavone contents in five *tempe* samples, based on wet weight

Name sample	Total phenolic content (mg/GAE/g)		mg Da/ 100 g		mg Ge/ 100 g		mg total isoflavone/ 100 g	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Taman Enquine (TE)	284.78 ± 22.47^{a}	7.89	1.98 ± 0.42	21.13	45.59 ± 16.43	36.04	44.87 ± 23.92^{ab}	53.32
Taman Universiti Indah (TUI)	252.56 ± 41.14^{ab}	16.29	2.35 ± 0.13	5.67	43.34 ± 2.12	4.88	43.71 ± 1.96^{ab}	4.50
Puchong (P)	233.64 ± 14.56^{b}	6.23	2.33 ± 0.07	2.80	27.64 ± 0.58	2.08	28.93 ± 0.35^{b}	1.21
Klang Lama (KLL)	246.19 ± 110.15^{ab}	44.74	2.41 ± 0.61	25.45	38.12 ± 6.78	17.78	35.65 ± 6.16^{b}	17.29
Selayang (S)	282.19 ± 16.41^{ab}	5.82	3.05 ± 0.38	12.51	56.87 ± 13.34	23.34	56.55 ± 12.23^{a}	21.62
Average	259.87 ± 22.62	8.71	2.42 ± 0.39	16.00	42.31 ± 10.68	25.24	41.94 ± 10.42	24.84

Values for total phenolic and isoflavone contents were averaged from four sample replicates and expressed as mean ± standard deviation.
Values with the different alphabets were ~~not~~ significantly different between the samples ($p < 0.05$)

CONCLUSION

The *tempe* produced from five cottage industries in Selangor showed that the macronutrients and total phenolic contents were similar. The total phenolic content was significantly correlated ($p < 0.01$) with total isoflavone content in all *tempe* samples. However, the mineral and isoflavone contents in the present study were lower compared to previous studies. Future study should collect and analysed more *tempe* samples from different locations in order to get more representative data.

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