Effect of two production methods on macro nutrient and isoflavone-aglycone composition in tempeh produced by household industries

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Abstrak

Latar belakang: *Tempe telah digunakan sebagai sumber gizi dan makanan yang menyehatkan. Pengrajin tempe memproduksi tempe dengan metode yang berbeda. Perbedaan metode dapat menyebabkan perbedaan mikrob selama fermentasi tempe dan komposisi kimia tempe. Oleh karena itu pengaruh metode yang berbeda terhadap kandungan gizi makro dan komponen aktif seperti aglikon-isoflavon pada tempe perlu dievaluasi.*

Metode: *Disain penelitian adalah penelitian lapangan dan analisis di laboratorium. Sampel tempe diambil dari dua pengrajin tempe di Bogor yang mengaplikasikan dua metode berbeda pada bulan Maret 2011 dan disimpan beku sebelum dilakukan analisis. Analisis terhadap kandungan gizi makro dan aglikon-isoflavon dilakukan pada bulan April-Juli 2013 dengan metode AOAC.*

Hasil: *Kecuali untuk kadar abu, berdasarkan berat basah, semua gizi makro dan komposisi aglikon-isoflavon tempe diproduksi dengan metode A sedikit lebih tinggi dari metode B. Namun berdasarkan berat kering, tempe yang diproduksi dengan metode B mengandung kadar gizi makro dan aglikon-isoflavon lebih tinggi dari tempe yang diproduksi dengan metode A, kecuali karbohidrat.*

Kesimpulan: *Perbedaan metode produksi tempe berpengaruh terhadap kandungan gizi makro dan aglikon-isoflavon. Namun demikian, tempe dari kedua pengrajin yang mengapliasikan metode yang berbeda dapat digunakan sebagai sumber gizi makro dan aglikon-isoflavon yang baik.* ***(Health Science Indones 2013;2:69-73)***

Kata Kunci: *Tempe, Gizi makro, Aglikon-isoflavon, mikrob*

Abstract

Background: Tempeh was used as nutrition source and healthy food. Tempeh household industries apply different production methods. Those different could lead to different microbial composition and hence different chemical composition of tempeh. Therefore it is necessary to evaluate the effect of different tempeh production methods on the content of macro nutrient and active compound such as isoflavone-aglyconee in tempeh.

Methods: The design of the study was field study and laboratory analyses. Samples of tempeh were collected from two tempeh household industries in Bogor, who applied different production methods in March 2011 and kept frozen before analyses. Analyses of macro nutrient and isoflavone-aglycone were carried out in the laboratory from April-July 2013 using AOAC methods.

Results: Except for ash content, on wet bases, all macro nutrient and isoflavone-aglycone composition of tempeh produced by method A was slightly higher than method B. However based on dry weight, tempeh produced by method B contained higher macro nutrition and isoflavone-aglycone than tempeh produced by method A, except carbohydrates.

Conclusion: Different tempeh production methods affected macro nutrient and isoflavone-aglycone content. However, tempeh from two household industries applying different method could be used as good sources of macro nutrient and isoflavone-aglycone. ***(Health Science Indones 2013;2:69-73)***

Key word: Tempeh, isoflavone-aglycone, macro nutrient, microbial

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Tempeh, especially soybean tempeh is the main nutrition source in Indonesia. Tempeh is rich of digestible protein, fat and carbohydrates. Tempeh is also containing active components, like isoflavones. So tempeh not only used as nutrition source, but also used as healthy food.[1] Until now tempeh used for nutritional sources and tempeh for active component source is undifferentited. Generally assumed, that the nutrient content of tempeh is similar regardless production methods, except for taste and texture.

Based on previous research, in Bogor there are two methods of tempeh production applied in household industries, production method A and B.[4] Sensory evaluation on those tempeh showed that the bitterness intensity of both tempeh was different.[2] Microbial diversity involved in both fermentation tempeh also different, including molds,[3] yeasts,[4.5] and bacteria,[2,3,4.5] especially lactic acid bacteria (LAB),[5] although they used similar soybeans and starter culture .[2,4]

Microbes function in fermented foods is to breakdown a complexes components into simpler components.[6] The diversity of microbes in tempeh fermentation may affect the nutrient content of tempeh produced, especially the macro nutrients (protein, fat, carbohydrate, ash, moisture, and energy content). Until now, the effect of tempeh production methods with different microbial diversity on nutrient content of tempeh has never been reported.

Isoflavones soybean also hydrolyzed during tempeh fermentation. Isoflavone-glycoside in soybean was hydrolyzed resulting isoflavone-aglycone (daidzein, genistein, glycistein).[7] isoflavone-aglycone in tempeh are more effective and efficient compared to isoflavone-glycoside on menopause symptoms treatment because they are more digestible and having higher antioxidant activity.[8]

Lactobacillus delbrueckii subsp *delbrueckii* KCTC 1047 is completely hydrolyses 50 mg^{-1} isoflavone-glycoside to isoflavone-aglycone in 30 minutes.[7] Based on the diversity analysis during tempeh production by method B, *Lactobacillus delbrueckii* subsp *delbrueckii* strains found in very high abundance, but this strain was not found during tempeh production by methods A.[5] *Lactobacillus delbrueckii* subsp *delbrueckii* was found in tempeh production method B, hence it is possible that the activity of this lactic acid bacteria increase isoflavone-aglycone content in tempeh produced by method B, but this needs to be proven.

This research aimed to evaluate the effect of different tempeh production methods on macro nutrient (protein, fat, carbohydrate, ash, moisture and energy content) and isoflavone-aglycone (daidzein, genistein, glycistein) composition.

METHODS

The design of the study was field study and laboratory analyses. Samples of tempeh were collected from two tempeh household industries in Bogor, who applied different production methods in March 2011 and kept frozen before analyses. Samples were taken at T5 stage of tempeh production line.[4] Analyses of macro nutrient and isolavone-aglycone using AOAC methods were carried out in the laboratory of Indonesian Agricultural Postharvest Research and Development Ministry of Agriculture Republic of Indonesia in Bogor from April-July 2013.

Sampling and Samples for Analysis

Sampling methods and samples for analysis were similar to the previous study,[4.5] involving two tempeh household industry that has been explored previously hereinafter referred to respectively tempeh production methods A and B.[4,5] Briefly, tempeh production method A applied just one time boiling step, soaking of un-hulled soybean, used subcultured commercial starter culture as inoculum and packaged in polyetylene plastic bag before incubation, while method B applied two time boiling step, soaking of de-hulled soybean, used commercial starter culture without sub-culturing and packaged in polyetylene plastic bag after incubation.[4] For this study, the samples were taken only after fermentation completed (fresh tempeh). All samples were frozen before analysis. Sampling was done twice from two cycle tempeh production with one day interval.

Analysis Procedures

Proximate analyses of fresh tempeh was done according to AOAC methods.[9] The parameters analyses included moisture, crude protein, lipids and ash content, while carbohydrate and energy were obtained by difference.

Analysis of isoflavone-aglyconee was done using by HPLC.[9] of Varian 940 LC with the stationary phase μ-Bondapack C 18 column and mobile phase acetonitrile : methanol : buffer ammonium acetate at pH 4,6 (rasio of 10: 50: 40 v/v) and the flow rate of 0,5 ml min^{-1}.

Samples was hydrolyzed on 0.1 mol HCl/L at 98^{o} C for 2 h, extracted with acetonitrile, and filtered through number 42 filter paper (Whatman). Then sample dried by using a rotary evaporator at 50^{o} C. The residue was dissolved in 100% ethanol. For analysis, extracted samples were injected at 40ml volume and detected at 261 nm. Peaks were identified based on retention time and compared with standard. Calculation of isoflavones was done based on standard curve. All analysis was done in duplicate.

RESULTS

Macro nutrient composition of both fresh tempeh produced by two tempeh household industries with different production methods (methods A and B) are presented in Table1.The macro nutrient composition of both tempeh were different. Table 1 shows the moisture content of tempeh produced by the method B was 21% higher than that of method A. On wet bases, except for ash content, fat, protein, carbohydrate contents and energy in 100 gram fresh tempeh produced by method A were higher than tempeh produced by the method B, but not for dry weight bases. Based on dry weight in 100 gram fresh tempeh, only carbohydrates of tempeh produced by method A was higher than tempeh product by method B (Table 1). All other macro nutrients were higher in tempeh produced by method B.

Table 1. Macro nutrient composition of fresh tempeh

Macro nutrient	Method of tempeh production		Difference (%)
	A	B	
Moisture (%)	33.57	40.64	21
Based on wet weight (100g)			
Fat (g)	20.1	20.0	1
Protein (g)	17.9	16.9	6
Ash (g)	0.9	1.0	14
Carbohydrates (g)	27.6	21.5	22
Energy (Kcal)	380.8	350.5	8
Based on dry weight (100g)			
Fat (g)	30.3	33.7	11
Protein (g)	26.9	28.4	6
Ash (g)	1.3	1.6	27
Carbohydrates (g)	41.5	36.2	13
Energy (Kcal)	573.2	590.4	3

Isoflavone-aglycone composition of both fresh tempeh produced by two tempeh household industry are presented in Table 2. The composition of isoflavone-aglycone (daidzein, genistein, glycistein) and total isoflavone-aglycone of both fresh tempeh produced by two tempeh household industries with different production methods was different. Table 2 shows on the wet bases, the isoflavone-aglyconees content of tempeh produced by the method A was higher than that of method B. Each isoflavone-aglycone (daidzein, genistein, glycistein) and total isoflavones-aglycone in fresh tempeh produced by method A was higher by 3,5,1 and 3% respectively than that of fresh tempeh produced by the method B. Similar to macro nutrient, on dry bases, isoflavon-aglycone content of tempeh produced by the method B was higher than tempeh produced by 8,6,11 and 9% for daidzein, genistein, glycistein and total aglyconees isoflavones respectively than those in tempeh produced by the method A (Table 2).

Table 2. Composition isoflavone-aglycone of fresh tempeh

Isoflavone-aglycone	Method of tempeh production		Differences (%)
	A	B	
Moisture (%)	33.57	40.64	21
Based on wet weight (100g)			
Daidzein (mg)	62.0	60.0	3
Geniestein (mg)	6.3	6.0	5
Glysistein (mg)	19.2	19.0	1
Total (mg)	87.4	85.0	3
Based on dry weight (100g)			
Daidzein (mg)	93.4	101.2	8
Geniestein (mg)	9.4	10.0	6
Glysistein (mg)	28.8	31.9	11
Total (mg)	131.6	143.1	9

DISCUSSION

Tempeh is a food that used as macro nutrients sources.[1] Protein, fat and carbohydrates and energy content of tempeh is high enough. Based on the nutrient composition of both tempeh in the current research, it are indicated that consumption of 100 grams of fresh tempeh contribute 20 to 20.1 grams of fat, 16.9 - 17.9 grams protein and 21.5 to 27.6 grams carbohydrates with energy 350.5 to 380.8 Kcal. Tempeh also contained minerals with total mineral of 1.3 to 1.6 grams in 100 grams fresh tempeh as (Table 1).

Both fresh tempeh used in this research was made from the same brand soybean and used similar starter culture, but the moisture and macro nutrients content of both tempeh were different. On dry bases, protein and fat content of tempeh produced by method B was higher than method A (Table 1). Higher protein and fat content of tempeh produced by the method B may indicate lower proteolytic, lipolytic activities and subsequent bioconversion of fatty acids and amino acids by microbes in tempeh production method B than method A. This may result in lower protein and fat loss in tempeh produced by method A was higher than method B, and protein as well as fat content of tempeh produced by method A was lower than method B on the dry bases (Table 1). Production methods and diversity of microbes during fermentation of both tempeh and tempeh product were different.[2,3,4,5] The difference of microbial diversity will lead to different of proteolytic, lipolytic and glycolitic activities, and further also different rate of bioconversion fatty acid, amino acid and simpler sugar to other component.[6,9] Those could lead to different composition of macro nutrient as shown in the present research.

Microbial activity on protein and fat resulted flavor components.[6] According to Kranenburg[10] LAB contribute to production aroma and flavor during fermentation via bioconversion amino acid. Different production method of tempeh caused the difference in diversity and abundance of LAB[5] and also the intensity of bitterness.[2]

Regardless the difference in macronutrient and isoflavone-aglycone content of tempeh produced by two different methods, tempeh is a good source of macro nutrient. In Indonesia tempeh used as protein source[1] for substitution meat, fish and other animal protein. Tempeh also known as healthy food.[1] Tempeh contains more digestible isoflavones and used for menopause symptoms treatment.[8] Based on data obtained from the present research, consumption of 100 grams of fresh tempeh contribute to 60 - 62 mg daidzein, 6 - 6 mg genistein,19 – 19.2 mg glycistein and isoflavone-aglycone total 85 – 87.4 mg (Table 2). This result higher than isoflavone-aglycone content in the yellow-soybean and black-soybean tempeh on previous research.[8] According to Nakajima,[8] composition of the isoflavone-aglycone in 100 grams wet yellow-soybean tempeh are 8 mg daidzein, 0.5 mg genistein,7.2 mg glycistein and isoflavon- aglycone total 15.7 mg, and for black soybean tempeh are 6.8 mg daidzein, 0.7 mg genistein, 9.9 mg glycistein and isoflavone-aglycone total 17.4 mg after 24 hour fermentation. The present results confirmed that tempeh regardless the production methods could be used as healthy food with high isoflavones content.

Composition of isoflavones in 100 gram of both tempeh were different Bases on dry weight, composition of all isoflavone-aglycone tempeh produced by method B were higher than tempeh produced by method A (Table 2). This data indicated that hydrolysis of soybean isoflavone-glucosides (genistin, daidzin, and glycitin) to be isoflavon-aglycone (genistein, daidzein and glycitein) in tempeh produced by method B higher than method A. *Lactobacillus delbrueckii* subsp *delbrueckii* strains found is abundantly during fermentation tempeh by method B and not found during tempeh production by methods A[5]. According to Choi,[7] *Lactobacillus delbrueckii* subsp *delbrueckii* KCTC 1047 completely hydrolyzed 50 mg^{-1} isoflavone-glycoside to isoflavone-aglycone in 30 minutes. *Lacbacillus delbrueckii* subsp *delbrueckii* found during fermentation tempeh by method B may be able to hydrolyze soybean isoflavone-glucoside to isoflavone-aglycone, but this has to be proven in the future study.

In conclusion, different tempeh production methods affected macro nutrient and isoflavone-aglycone content. This may correlate with the different microbial diversity. However, tempeh from two household industries applying different method could be used as good sources of macro nutrient and isoflavone-aglycone.

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