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β-Conglycinin Content Obtained from Two Soybean Varieties Using Different Preparation and Extraction Methods

MEILINAH HIDAYAT[1*], MUCHTAN SUJATNO[2], NUGRAHA SUTADIPURA[2], SETIAWAN[2], AHMAD FARIED[2]

[1]Department of Nutrition, Faculty of Medicine, Maranatha University, Jalan Prof. Drg. Suria Sumantri Street 65, Bandung 40163, Indonesia

[2]Faculty of Medicine, Universitas Padjadjaran, Eijkman Street 38, Bandung 40161, Indonesia

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Soybean is a good source of protein. It has two major fractions, β-conglycinin (7S) and glycinin (11S). β-conglycinin's function was known to suppress food intake, and this effect may be due to stimulating endogenous cholecystokinin (CCK) release. The aims of this study were to determine the highest content of total β-conglycinin and β-conglycinin sub unit-β level obtained from two varieties of soybean i.e. *Wilis* and *Detam 1* varieties using different preparation and extraction methods. These two soybean varieties were prepared into tempeh. Then the seed and tempeh were extracted using Deak and Panthee methods. There were six extracts analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and coomassie brilliant blue (CBB) staining. The result was shown that *Detam* variety and raw seed contained the highest total β-conglycinin level. And Panthee method was the best method for extraction of total β-conglycinin, while Deak method was the best method for extraction of β-conglycinin subunit-β.

Key words: *Detam 1* soybean, *Wilis* soybean, Deak method, Panthee method, SDS PAGE, CBB staining

INTRODUCTION

Soybean is a source of protein and amino acids for human and animal feeds in the world. Many works have been done on structure, biosynthesis and genetics of the soybean proteins (Nielsen 1984; Clarke & Wiseman 2000).

Soybean storage protein has two major fractions, β-conglycinin (7S) and glycinin (11S), accounting for more than 70% of the total proteins. β-Conglycinin is a trimer with subunits α, α', and β, with a M.W. (molecular weight) of 180 kDa. Glycinin is a hexamer with a M.W. of 360 kDa, consisting of acidic and basic subunits (Kitamura 1995).

Soybean protein contained 8 essential amino acids. Soybean contained higher amino acids than that of animal products. Protein from legume groups, like soybean, is absorbed slower than protein from livestock (Symolon 2004). Nutritional study showed that soybean protein produced higher thermogenic and satiety effect than that of carbohydrate. This fact made soybean as a good nutrition source for treating obesity (Mc Carthy 2000). However soy protein is not a perfect protein because of its low level of the sulfur-containing essential amino acids such as methionine and cysteine. Glycinin has three to four times more S-containing amino acids (particularly methionine) than that of β-conglycinin (Kitamura 1995). On the other hand, soybean protein contains β-conglycinin that may be important food components to control lipid accumulation in adipose tissues (Moran 2006).

Based on many studies, β-conglycinin's function was approved to suppress food intake due to releasing β-conglycinin peptone in the lumen stimulating endogenous Cholecystokinin (CCK) with direct acceptance to the intestinal cells (Nishi 2001; Nishi 2003a). The fragment from 51 to 63 of the β subunit (β 51-63) had the strongest binding activity to stimulate releasing CCK and appetite suppression (Nishi 2003b). CCK is an important hormonal regulator of the digestive process. The physiological actions of CCK include stimulation of pancreatic secretion and gallbladder contraction, regulation of gastric emptying, and induction of satiety. Therefore, by its functions which can stimulates CCK made β-conglycinin in soybean as one of the important therapy targets in obesity treatment (Liddle 1997; Little 2005).

There were many varieties of soybean in Indonesia. In this study we focused on 2 varieties of soybean i.e. *Detam 1* and *Wilis* varieties. *Detam 1* variety is a high quality soybean mostly cultivated in Balai Penelitian Kacang dan Umbi-umbian (Balitkabi) plantation in Malang, Indonesia. It contained higher protein level (45.36%) than that of other soybean varieties (MenTan 2008). So it was assumed that it also contained a high level of bioactive compound in the seed. As a comparison we used soybean *Wilis* variety, an Indonesian local soybean which is commonly planted by farmers in Indramayu, indonesia.

A study focus on the effect of different preparation method of soybean, like tempeh, to produce β-conglycinin was very limited. Fermentation process decreased the protein content of soybean but the amount of protein that can be absorbed was increased due to inactivation of

*Corresponding author. Phone: +62-22-2012186, Fax: +62-22-2017621, E-mail: mellahidayat@yahoo.com

antinutrition factors in soybean by heating during fermentation process (Hermana 1999; Buckle 2007). However, its correlation with β-conglycinin production was not clear yet.

There are several kinds of extraction methods to produce β-conglycinin. And in this study we focused on two methods of soybean protein extraction i.e. a Panthee method and a new simplified Deak protein fractionation procedure. The Panthee method is an extraction procedure to get higher glycinin level (Panthee *et al*. 2004; Delwiche 2007). And Deak method is the procedure that is possible to produce protein fractions (> 90% protein) which is rich in either glycinin or β-conglycinin by employing $CaCl_2$ and $NaHSO_3$ (Deak 2007). In this study, we consider to find out the best method to extract β-conglycinin especially subunit-β as one of therapy target in obesity treatment in the future.

The aims of this study were to determine the highest content of total β-conglycinin and β-conglycinin sub unit-β level obtained from two varieties of soybean i.e. *Wilis* and *Detam 1* varieties using different preparation and extraction methods analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and coomassie brilliant blue (CBB) staining.

MATERIALS AND METHODS

Soybean Variety. Two soybean varieties chosen in this study were *Wilis* and *Detam 1* varieties. The soybean *Detam 1* variety was planted in Balitkabi plantation in Malang, Indonesia. Productivity of *Detam 1* variety was 2.51 ton per acre, seeds were harvested in 84 days. This soybean had a yellow flesh seed covered with hard black skin. It contained 45.36% dry weight protein which was higher than that of other varieties. This variety was approved as a high quality soybean by Minister of Agricultural decree no 240/Kpts/SR.120/3/2008 date March 6th 2008 (MenTan 2008).

Wilis variety is an Indonesian local soybean which is commonly planted by farmers in Indramayu, Indonesia. This soybean has a yellow flesh seed with yellow skin seed. It contains about 39% protein (dry weight).

Sample Preparation. The two soybean varieties were made into tempeh. Then the seed and tempeh from *Detam 1* and *Wilis* varieties were extracted using Deak and Panthee procedures. So that there were 6 extracts of soybean examined in this study i.e. (i) Protein extract of *Detam 1* Soybean seed Deak Method; (ii) Protein extract of *Detam 1* Soybean seed Panthee Method; (iii) Protein extract of *Detam 1* Soybean tempeh Panthee Method; (iv) Protein extract of Wilis Soybean seed Panthee Method; (v) Protein extract of Wilis Soybean tempeh Panthee Method; (vi) Protein extract of *Detam 1* Soybean skin seed Panthee Method.

Fermentation of Soybean. Procedure steps to make Tempeh was conducted in 8 steps process i.e. boiling, peeling of skin seed, soaking, washing, steaming, inoculating, packaging and stewing. As many as 500 g *Detam 1* and *Wilis* soybean seeds were boiled. Then the skin seed was peeled and soaked in an acid condition (pH 4.0-5.0). The skin of the seed was also keep to be extracted of their protein. Then the soybean seeds were washed so that they were not sleek. The seeds were steamed until they were soft and wellcooked.

Fungal tempeh inoculum or *laru* was inoculated 1 g for 1 kg of the seeds and they were mixed thoroughly. After the seeds were packed in plastic bags, they were incubated at room temperature (20-37 °C) for 2 days (Hermana 1999; Santoso 2003). It was produced 6,500 g of Tempeh from 500 g of raw seeds.

Protein Extraction Panthee's method. Sample of 10 g was grounded in cool water (20 °C) using Knifetec 1,095 sample mill for 20 s. It produced soybean flour with relatively uniform particle size. Soluble protein was extracted for 1 h at room temperature from 20 to 25 °C (68 to 77 °F). One gram of soybean flour was stirred in 0.2M Tris HCl buffer pH 8.0 with ratio of 1:15 (w/v) contained 0.1 M β-*mercaptoethanol.* The mixture was centrifuged at 10.000 x g for 10 min at 4 °C. Upon removal of the fat layer, as much as 1 ml of aliquot layer or supernatant was taken from each sample. The proteins in the crude extract sample were dissociated by adding an equal volume of both 5% SDS and 0.1 M β-*mercaptoethanol* solutions and then warmed at 44.5 instead of 45 °C in a waterbath for 10 min (Panthee *et al*. 2004; Delwiche 2007).

Deak's Method. Defatted soy flour of 100 g was extracted using deionized water with ratio of 1:15 (w/v) at pH 8.5 adjusted with 2 N NaOH. The slurry was stirred for 1 h and centrifuged at 14,000 g and 15 °C for 30 min. The protein extract (first protein extract) was decanted. The extract was then added with sufficient $NaHSO_3$ and $CaCl_2$ to obtain concentration of either SO_2 and Ca_2+ to 5 mM and the pH was adjusted to 6.4 with 2 N HCl. The slurry was stored at 4 °C for 12-16 h. The slurry was centrifuged at 14,000 g for 30 min at 4 °C. A glycinin-rich fraction was obtained as the precipitated curd, which was neutralized and treated as described on Wu procedure.

The supernatant (second protein extract) was adjusted to pH 4.8 with HCl. Then it was stirred for 1 h and centrifuged at 14,000 g and 4 °C for 30 min. A β-conglycinin-rich fraction was obtained as the precipitated curd and treated as described previously. The amount of supernatant (whey) was determined and sampled.

Freeze-drying steps were modified to evaporation process in cycling evaporator at 30 °C until the solution extracts become thick liquid. Samples were placed in sealed containers and stored at 4 °C up to analysis (Figure 1).

There are two steps in Deak's procedure i.e. D4C (in 4 °C) and DRT (in 25 °C). The Deak β-conglycinin-rich fraction D4C (the methods which performed in this study) comprised 23.1% of the solids, 37.1% of the protein and 37.5% of the isoflavones in the starting soy flour. Protein purity was > 80%. Their D4C method produced 85.6% β-conglycinin and 14.4% glycinin. The β-conglycinin subunit consisted of 27.3% subunit α' 38.0% subunit α, 34.7% subunit α (Deak 2008).

SDS-PAGE. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according