Effect of various solvent on the specific amino acids of black soybean (*Glycine soja*) sprout

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Abstract. The objective of this research was to study the effect of various solvent extractions on the specific amino acids as small peptide or free amino acids that was contained in the extract after removal of the macromolecule protein of black soybean sprouts. The experimental design of this research was randomized complete design with one factor, which was the three various solvent, *i.e*. hexane, ethanol and water. The black soybean seed was germinated for 36 h. The small peptide and free amino acids of black soybean sprout were isolated at 3 various of solvents extraction, and then the macromolecule proteins in the extracts were precipitated at the pH 4. The extracts of black soybean sprout after removal of the macromolecule protein were analysed by HPLC to determine the profile of amino acids for stimulation of insulin secretion. The result of this research showed that the extracts contained the small peptide and free amino acid for stimulation of insulin secretion. The best solvent extraction was water that was due to the content of Leu, Arg, Ala, Phe, Ile, and Lys of water extract was higher than hexane and ethanol extracts.

Keywords: *solvents, amino acids, insulin, black soybean, sprout*

1. Introduction

Soybean seed has popularly known as functional food for diabetes, it is due to its ability to reduce blood glucose. There were two hypoglycaemic components of soybean seed. The first component is trypsin inhibitor, however the consumptions of food that contain trypsin inhibitor may cause pancreatic hypertrophy. The second component is specific amino acids that are known as insulin stimulation. Amino acids may influence insulin secretion via a number of possible mechanisms, including generation of metabolic coupling factor, depolarization of the plasma membrane, or enhancement of mitochondrial function [11,3]. However the availability of amino acids in soybean seed naturally combined with each other to make long chains in the form of protein-macromolecule complex. The acceleration of amino acids in the form of macromolecule to be utilized by pancreatic β-cell for stimulation of insulin secretion may be lower than small peptide and free amino acids.

Seed germination increased protease activity that could degrade protein, hence the small peptide and free amino acid in soybean sprout may be higher than the original seed [1]. Based on in vivo and in vitro bio-assay, the specific amino acids were known as stimulation of insulin secretion such as Leucine/Leu, Lisoleucine/Ile [13,16,], Arginine/Arg [8,16], Alanine/Ala, Phenylalanine/Phe, Lysine/Lys [14,4,15]. Therefore the consumption of food that contains the specific amino acids as insulin stimulation may accelerate an increase in plasma insulin [12] which was useful for diabetes

therapy. The research had showed that soybean sprouts protein could increase stimulation of insulin secretion due to the specific amino acids, such as Leu and Arg were higher than the seeds [6]. The profile of amino acid for stimulation of insulin secretion in the water extract of local legumes sprout (winged bean/*Psophocarpus tetragonolobus*, velvet bean/*Mucuna pruriens*, cowpea/*Vigna unguiculata*, and soybean/*Glycine max*) had been determined in the previous research [7]. This research was conducted to know what the black soybean sprouts contain the small peptide and free amino acids for stimulation of insulin secretion, such as amino acids Leu, Arg, Ala, Phe, Ile, and Lys.

Amino acids are carboxylic acids which have amino group and functional or R group or side chain in the structure. Based on the side chain of amino acids are classified into amino acids with non polar or hydrophobic side chains, polar but neutral side chains, polar but negative or acidic side chains, and polar but positive or basic side chains [9]. There are amino acids for stimulation of insulin secretion that are classified into non polar side chain amino acids, such as Leu, Ile, Ala, and Phe. While the others are classified into basic amino acids, such as Arg and Lys. The objective of this research was to study the effect of various solvent extraction on the amino acids Leu, Arg, Ala, Phe, Ile, and Lys in the extract after removal of the macromolecule protein of black soybean sprout.

2. Material and methods

The black soybean was obtained from Research Center of Legumes, Malang, Indonesia. Chemical agents such as amino acids standard, OPA (Ortho Phentaldialdehyde) were purchased from Sigma, Fluka and Merck Chemical Co.

2.1. Preparation of the extracts of black soybean sprout in various solvent

Black soybean (*Glicine soja*) seeds were soaked for 8 h, and then germinated for 36 h. Germination of black soybean for 36 h was selected for producing soybean sprout. The black soybean sprout was dried at 50^{o}C and then was milled in order to be flour. The extracts of black soybean sprout were prepared by mixing the flour and 3 various solvents, that were hexane, ethanol or water at 40^{o}C, 15 minute and the ratio of the flour and solvent was 1:10. After centrifugation, the macromolecule protein in the extracts were precipitated at pH 4 and centrifugation. Then the extracts of black soybean sprout after removal of the macromolecul protein were analysed by HPLC to determine the profile of amino acids [6]

2.2. Preparation of sample and amino acids standar solution for HPLC analysis

Amino acids profile was analyzed by OPA (ortho phentaldialdehyde) method [2] with modification using HPLC (High Performance Liquid Chromatography). Ethanol and hexane in sample extracts were heated to evaporate the organic solvent. 5ml of water extract was added 5ml 12N HCl, while 5 ml of hexane and ethanol extracts were added 5ml 6N HCl, and then were hydrolyzed by autoclafe at 110^{o}C, 12 h. After that, the samples were cooled and neutralized by adding 6N NaOH. Add 2.5 ml 40 % Pb-acetate and 1 ml 15 % oxalate acid and then was fixed 50 ml with aquabides. Take 3 ml and filtered by millex 0,45 µm, and then mixed with OPA solution for 3 minute. The 30 µL sample solution was injected to HPLC.

Take 50 µL 2.5 ppm amino acid standard solution (Leu, Arg, Ala, Phe, Ile, and Lys) and then was mixed with 950 µL OPA solution for 3 minute. The 30 µL standar solution was injected to HPLC. The conditions of HPLC of the analysis were Column: Eurospere 100-5 C18, 250x4,6mm with precolumn P/N: 1115Y535; Eluen: A = 0.01M Acetic buffer pH 5.9; Eluen B = MeOH: 0.01M Acetic buffer pH 5.9:THF 80:15:5; λ Fluorescence: Ext = 340 nm; Em = 450 nm.

2.3. Experimental design and data analysis

The experimental design of this research was randomized complete design with 1 factor, which was the three various solvent, *i.e*. hexane, ethanol and water. The experiment was done with 2 replication. Statistical analysis by Analysis of Varian and Duncan Multiple Range Test with 0.05 significant level

3. Result and discussion

The analysis of amino acids profile of the sample of black soybean sprout extracts were preceded by preparation of sample to hydrolyse small peptide and liberate amino acids. After that the sample could be injected to HPLC. The amount of free amino acids in samples could be known by comparison of retention time of samples chromatogram with standard chromatogram of amino acids. Based on the chromatograms could be determined the amount of amino acids in the extract of black soybean sprout with the various of solvent after removal of the protein, that was seen at Table 1. The chromatograms of this analysis could be seen at Fig. 1a for chromatogram of amino acids standard and Fig. 1b, 1c, and 1d for chromatogram of sample that was extracted by solvent of hexane, ethanol, and water respectively.

Table 1. The amount of amino acids for stimulation of insulin secretion in the extract of black soybean sprout with the various solvent (hexane, ethanol, and aquadest) after removal of the protein.

Amino acids	The amount of amino acid (µg/ml of extract) *)		
	Hexane	Ethanol	Water
Alanine (Ala)	4.40[a]	30.80[b]	360.00[c]
Arginine (Arg)	1.40[a]	5.80[b]	614.40[c]
Phenylalanine (Phe)	1.80[a]	7.40[b]	192.00[c]
Isoleucine (Ile)	1.40[a]	8.00[b]	216.00[c]
Leucine (Leu)	2.20[a]	10.40[b]	312.00[c]
Lycine (Lys)	4.80[a]	15.20[b]	480.00[c]

*) The different alphabetic notation after the data in the table showed significant differences at the same row and the different column.

Data in Table 1 showed that the extracts of black soybean sprout after removal of the protein still contained amino acids for stimulation of insulin secretion, indicating that the extract that was the waste of protein isolation had the potency as the source of amino acids. Protein isolate is raw material of meat analog. Usually, the extract after removal of the protein in the processing of protein isolate as raw material of meat analog is not to be utilized. The extract is the waste of this processing. This research also indicated if the protein isolate as raw material of meat analog was made of germinated legumes, the extract would contain small peptide and free amino acids. That was due to germination could increase protease activity and then proteins are hydrolysed by proteases, resulting in several small peptides and free amino acids [1]. Hence, amino acids for stimulation of insulin secretion of the extract could be recovered and processed as the side product of protein isolation.

This research also showed that the best solvent extraction was water it was due to the content of Leu, Arg, Ala, Phe, Ile, and Lys of water extract was higher than hexane and ethanol extracts (Table 1). This phenomena indicated that the solubility of small peptides and free amino acids in the water solvent was higher than the hexane and ethanol solvent, although some amino acids (Leu and Ile) are known as non polar amino acids. That may be due to the mechanism of free amino acids in the extracts was trapped in the small peptide that was soluble in the water solvent. The research had been showed model the amino acid solubility in water with very good precision, including L-tyrosine, L-leucine, L-aspartic acid, L-tryptophan, L-glutamic acid, L-alanine, DL-alanine, DL-valine, L-phenylalanine, DL-serine, L-proline, L-serine, and glycine [5]. Hydrophilic peptides containing more than 25% charged

residues of amino acids and 25% hydrophobic amino acids are usually soluble in water or aqueous buffers. Peptides containing 50% and more hydrophobic residues might be insoluble or only partly soluble in aqueous solutions. Researchers should always attempt to dissolve peptides in sterile water first, especially when the peptide contains less than five residues of amino acids. Peptide solubility is also effected by electron acceptor and electron donor solvent properties [10].

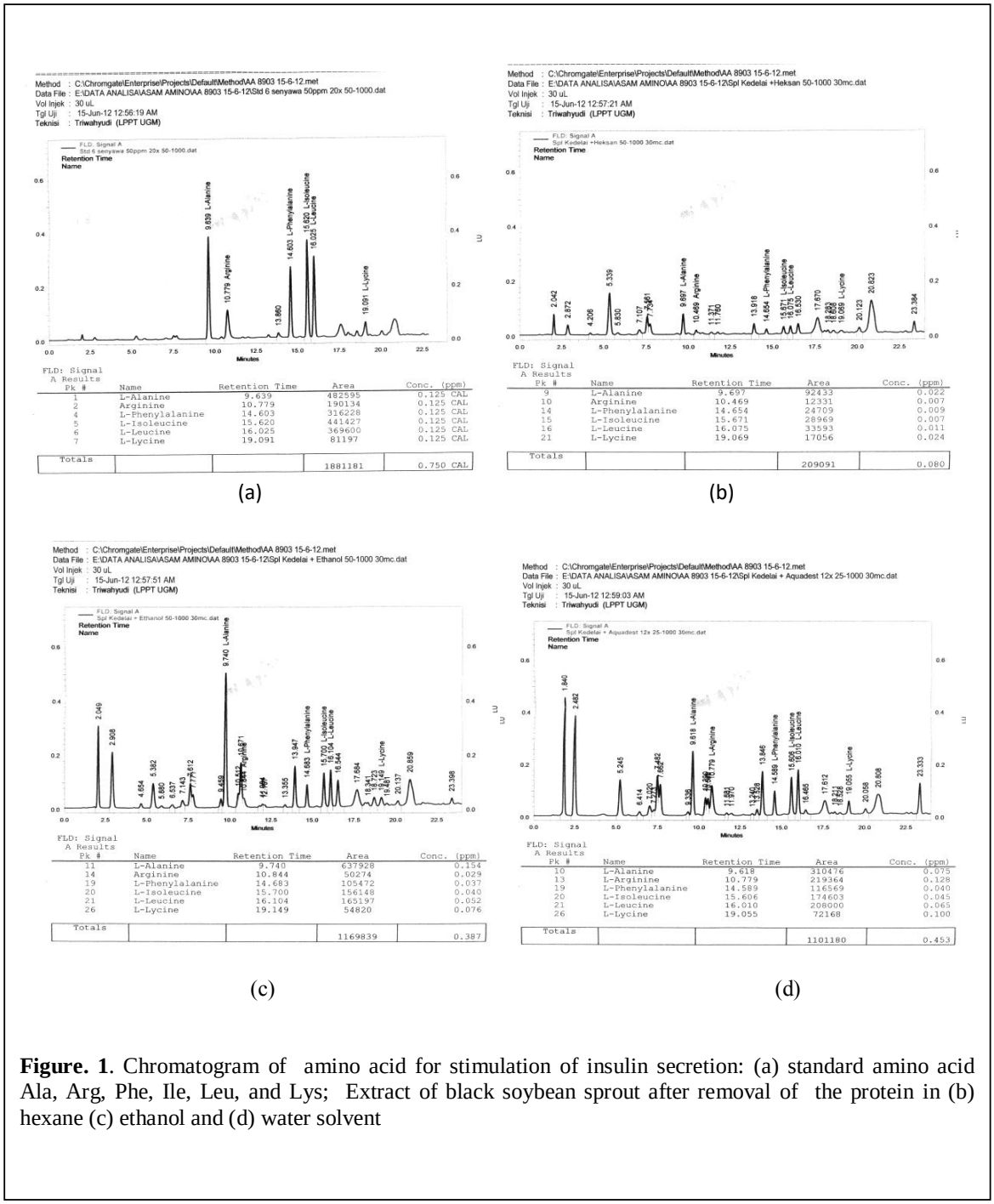

Figure. 1. Chromatogram of amino acid for stimulation of insulin secretion: (a) standard amino acid Ala, Arg, Phe, Ile, Leu, and Lys; Extract of black soybean sprout after removal of the protein in (b) hexane (c) ethanol and (d) water solvent

stimulation of insulin secretion was water that was due to the content of Leu, Arg, Ala, Phe, Ile, and Lys of water extract was higher than hexane and ethanol extracts. Hence, the extract could be processed as the side product of protein isolation which was useful for diabetes therapy.

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