EDAMAME FLOUR (Glycine max (L) merill) AS SOURCE OF OLIGOSACCHARIDES AND DIETARY FIBER: CHARACTERISATION OF PHYSICAL, CHEMICAL AND PHYSIOLOGIS

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EDAMAME FLOUR (*Glycine max* (L) merill) AS SOURCE OF OLIGOSACCHARIDES AND DIETARY FIBER: CHARACTERISATION OF PHYSICAL, CHEMICAL AND PHYSIOLOGIS

SUMMARY

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SUMMARY

I. INTRODUCTION

Currently food products just not only are requested to have taste, appearance and the contains of nutrients to fulfill basic needs of the body, but directed on the ability providing benefits in body health and if possible capable in preventing of diseases. The concept of food formulations be directed to produce products that gives health benefits known as functional food, that's the food and components food that provides a health benefit beyond basic nutrition (IFT, 2005). Examples may include conventional food, fortified, enriched. Functional food in principle contain natural material components includes dietary fiber, oligosachharides, sugar alcohol, an unsaturated fatty acid, poliphenol, isoflavone and others.

Processing can change dietary fiber and oligosachharides contents, this is described by previous research. Kutos *et al*., (2003); Rehinan *et al*., (2004) reported that thermal processing did cause decreasing in total dietary fiber and water insoluble fiber in legume. Sat and Keles, (2002); Ruperez, (2005) reported that during soaking and thermal processing there were a decreasing of oligosaccharides content in legume, such as raffinose, stachyose and verbascose.

Based on this consideration, it was hypothised that edamame is one of alternative sources of dietary fiber and oligosaccharides. Considering that until now no study about exploring edamame as a new source of dietary fiber and oligosaccharides, then the researcher interested to study this case on edamame flour. This research studied the development of products on edamame flour-based

which rich in dietary fiber and oligosaccharides as functional food. The products was obtained by the removal of fat (defatted) followed the removal of protein (deproteinized) resulted in defatted and deproteinized edamame flour. In the form of flour , this product can be used for various formula of food. Hence, examined the chemical and functional properties of defatted edamame flour and defatted & deproteinized edamame flour. To study the physiological effect of the product, it was carried out by a bioassay with *Sprague Dawley* rats as the model. In this bioassay the hypolipidemic and hypoglycemic properties of defatted & deproteinized edamame flour were studied. The digesta properties including the physical properties (weight) and chemical properties (pH, water level, SCFA) of the digesta were also determined. As a comparison conventional flour product namely freezing edamame flour and canning edamame flour were also studied.

II. LITERATURE REVIEW

Food processing affects dietary fiber. It is shown by Kutos *et al*., (2003) that the heat processing causes decreasing in insoluble fiber and total dietary fiber in legumes. Increasing consumption of high dietry fiber both water soluble fiber and water insoluble fiber give influence that favorable to health and reduce the risk of some diseases (Mc Intosh, 2001).The increase intake of water soluble food fiber significantly reduce cholesterol and glucose concentration and corony heart diseases (Anderson *et al*., 2009, Slavin, 2013).

Not all of dietary fiber have same effectiveness in lowering cholesterol level. In addition, the number of dietary fiber given very powerful on decreasing rate of cholesterol in experiment animals and human volunteer. Anderson (1994) reported that ability of some dietary fiber to bind the acids bile and bring out the bile acids with feces led to changes in normal enterohepatic circulation. Increasing losses of bile acid into feces will decrease the bile resircullation to the liver and the liver was promoted to synthese addition of bile acid using cholesterol taken from the blood. It was one of the mechanism of reduction cholesterol of dietary fiber.

Dietary fiber also have play important roles in decreasing of blood glucose levels. Study about mechanism of decreasing blood glucose serum *in vitro* reported by Ou *et al* (2001) that dietary fiber lowered postprandial serum glucose level at least by three mechanisms are (1) dietary fiber increase the viscosity of smooth intestinal juice and hinder diffusion of glucose, (2) dietary fiber binds glucose and decrease the concentration of available glucose in the small intestinal, (3) dietary fiber retard α-amilase action through capsuling starch and the enzyme and might directly inhibit the enzyme.

Dietary fiber and resistant starch fermented by the anaerobic bacteria in ceacum and large intestine produce SCFA. According to Rose *et al*. (2007), SCFA is important as source of energy to colonicytes, maintain the integrity of colon barier function. Another possibility SCFA give contribution to decreased of cholesterol. This might because the influence of acetic and propionic acids

reached liver affecting liver glucose metabolism or cholesterol synthesis (Wolever,1991; Hara *et al*., 1998; Maki and Rain, 2011).

Among food modification, intake of dietary fiber have an important roles in management of syndrome metabolics through distinctions source of dietary fiber by chemical structures, physical characteristics, and fermentable characteristics in the intestines. The difference type of dietary fiber were reported among some of them able to control body weight, glucose homeostatis and lipids, sensitivity of insulin and squeeze few inflammatory maker involved in patogenesis of metabolics syndrome (Galisteo *et al*., 2008).

Edamame is a potential health food. Edamame is one of very good food materials for diabet patient, because able to reduced blood glucose levels (Mullet, 2007). Beside this, edamame also have some potention in health such as to prevent cancer effects, vascular diseases, osteoporosis (Anderson *et al*.1997 and Sirtori *et al*., 2001 in Sirisombon *et al*., 2007).

III. Research Methods

3.1. Material dan Instrument Research

3.1.1. Material Research

Raw material used in this research is edamame soybean of Ryokoh variety obtained from PT Mitra Tani 27 Jember, and soybean was Baluran variety from local farm in Mangli subdistrict Jember Residence East Java. Chemical materials which used in sample analyzing is pro analysis grade obtained from

Merck(Germany) and standard from Sigma-Aldrich (USA). Reagensia for proxymate analysis, food fiber analysis, determining of oligosaccharides level, reagen GOD-POD Kit for analysis of determining blood glucose levels dan profile lipids (DiaSys Diagnostic System Germany) and distillation water (aquadest).

Thirty experimental male *Spaque Dawley* rats of 2 months age with weight of 170-200 gr were obtained from Balai POM Jakarta. Compotition of feed diet referred to the formula of *American Institute Nutrition* (AIN 93) (Reeves *et al*., 1993).

3.2. Stages of Research

This research includes stages as follows:

Stage 1. Prepared products and determination of total dietary fiber and oligosaccarides, defatted edamame flour and defatted & deproteineized edamame flour, raw edamame flour, freezing edamame flour and canning edamame flour.

Stage 2. Determination chemical and functional properties of defatted edamame flour and defatted&deproteineized edamame flour, raw edamame flour, freezing edamame flour and canning edamame flour.

Stage 3. Determination physiologial effects of defatted edamame flour and defatted&deproteineized edamame flour, freezing edamame flour and canning edamame flour with *in vivo* on *Sprague Dawley* rats on hypolipidemics, hypoglicemic properties, and the digesta properties.

3.3. Analysis Methods

1. Proxymate analysis (AOAC, 1995)
2. Dietary fiber analysis with Multi-enzyme Methods (Asp *et.al*, 1983).
3. Functional properties analysis :
    a. Water Holding Capacity (Suzuki *et.al*., 1996);
    b. Oil Holding Capacity (Caprez *et.al*., 1986)
4. Oligosaccharides level analysis (Black and Bgaley, 1976)
5. Lipids profile analysis
    a. Total Cholesterol (CHOD-PAP, Richmond, 1973)
    b. LDL Cholesterol (CHOP-PSP, Wieland & Siedal, 1983)
    c. HDL Cholesterol (CHOD-PAP, Eckal *et al*., 1977)
    d. Trigliserida (GPO-PAP, McGowan *et.al*., 1983)
6. Blood glucose analysis GOD-POP (Barham and Trinder, 1972)
7. The digesta properties
    a. Water content of the digesta (AOAC, 1995)
    b. Weight of the digesta (AOAC, 1995)
    c. Determination of pH
    d. Determination of SCFA (Zaron *et al*., 1997

IV. Result and Discussion

4.1. Chemical Properties and Dietary Fiber of Edamame Flour and Soybean Flour.

4.1.1. Chemical Property

Edamame flour and soybean flour are rich protein level, but protein level of edamame defatted&deproteinized (EDFP) flour is higher than soybean flour. On treatment removal of fat (*defatted*) cause decreasing protein level from 36,37% on raw edamame (RE) to 29,91 % on edamame defatted (EDF) flour, so on soybean defatted flour up to 28,91 %. It is presumable because there is amino acid fat-soluble dissolved in pertoleum benzene solution. Some amino acid fat-soluble are alanine, valine, leucine, isoleucine, proline, fenilalanine, tryptopan dan metionine (Silva *et al*., 2009).

After defatted process, followed by removal of protein (deproteinized) which affect decreasing in protein level on EDFP flour was 17.03% and soybean defatted&deproteinized flour was 15.77 %. Decreasing of this protein level because process of deproteinizing used NaOH of pH 9 solution for solute protein in edamame flour dan soybean flour. Beside this, possibility also protein soluble (some amino acids) solute in fat (non polar) and dilute in non polar soluter is tolune used in deproteinized process.

4.1.2. Dietary Fiber

The development of edamame as a fuctional food was done with removal of fat and protein was expected to increase complex carbohydrate content. This

process affect to total dietary fiber level. Edamame defatted flour and defatted & deproteinized edamame flour have high dietary fiber concentration. Total dietary fiber of defatted edamame flour and edamame defatted & deproteinized flour are 39,91 % and 43,16 %, respectively. Total values of dietary fiber was higher than total raw edamame dietary fiber. Advance of total dietary fiber on defatted edamame and defatted&deproteinized presumable because reducing of amount of fat and protein, so increasing procentage of dietary fiber. It can be noted that defatting process dan defatting & deproteinizing able to increase total dietary fiber, insoluble fiber and soluble fiber.

Freezing edamame flour and canning edamame flour have total dietary fiber of 27,98 % and 25.20 %, this values lower than total dietary fiber of raw edamamme flour of 30,19 %. Of this study can be learned that processing of freezing and canning decreasing total dietary fiber level. Decreasing total dietary fiber level as the effects of decreasing insoluble fiber and soluble fiber level. Kutos, *et al.,* (2003); Rhehinan *et .al.*, (2004) reported that thermaly processing, autoclaving decreased insoluble fiber level due to decreasing of celullose, hemiselulosa and lignin contents.

4.2. Functional Property of Edamame Flour and Soybean Flour.

4.1.1 Water Holding Capacity (WHC)

WHC of defatted & deproteinized edamame flour and defatted & deproteinized soybean flour have higher values than raw and defatted edamame

flour. It is presumable because on process of removal of protein dissolved non polar amino acid, so give opportunities to hydrophilics amino acid absorbed and tied maximum water (Lin *et al*., 1974 in Aslam *et al*. 2013). The hydrofilics amino acid including lycine, serine, threonine, cysteine, tyrosine.

Freezing edamame flour and canning edamame flour have WHC values higher than WHC of raw edamame flour. Its presumbable because processing affects on the exposure of hydrophilics groups in protein molecules to outer environment which facilitates the maximum absorption of water on the surface of protein (Lin *et al*., 1974 in Aslam *et al*., 2013).

4.2.2 Oil Holding Capacity

Defatting & deproteinizing increase OHC values of defatted & proteinized edamame and soybean flour. Increasing of OHC might be due to the decreasing of hydrophobic properties of protein flour as reported by Odebole and Lawal (2004). It is also possible that using of toluene in the process of defatting might disolve the non-polar amino acids and decrease the hydrophobicity of the flour.

4.3 Determination of The Oligosaccharides Level of Edamame Flour

FOS content of defatted edamame flour were similar with defatted & deproteinized edamame flour. Rafinosa levels of defatted edamame showed was the lowest among the other kind of flour studied. While defatted & deproteinized

edamame had rafinosa levels of 1,619 %. Both types of flour were lower than raw edamame flour, it was probably due to the thermal process during preparation of the flour.

Freezing edamame flour has FOS 1.57 % and rafinosa 0,662 % .This value is greater compared with canning edamame flour which have FOS level of 0,168 % and rafinosa of 0,197 % . The lower value of rafinosa of the canning product can be due to the thermal processing during canning

4.4 Physiologyc Effect of Edamame Flour on Lipid Profile

4.4.1. Body Weight

In rats with hypercholesterolemia and was given a standar diet, the amount of intake quite high but decreasing in body weight. Weight loss in the diabetic rats reflected the lower uptake of glucose to the addipose tissue. Therefore to fullfil the energy requirement fat and protein of the muscle were hydrolised through proteinolysis and lipolysis resulted in decreasing body weight.

4.4.2. Lipid Profile Serum

Intervention diet of defatted edamame, defatted & deproteinized edamame, freezing edamame, canning edamame give influence significantly to total cholesterol, LDL cholesterol and triglycerides ($p<0.05$) of rats with hypercholesterolemia and hyperglicemia. The defatted& deproteinized edamame diet more effective to reduce total cholesterol, LDL and triglycerides compared

with other edamame. Defatted & deproteinized edamame diet decreased total cholesterol, LDL and triglycerides of 55,37 %, 39.37 % , 42,95 % respectively, increasing of HDL cholesterol by 181,47 %. Defatted edamame diet decreasing total cholesterol, LDL, triglyceride by 50.46 %, 34,13 %, 34,08%, respectively. increasing HDL cholesterol of 95,25 %, compared freezing edamame diet able to reduce total cholesterol, LDL, triglyceride by 40,91 %, 13,08 %, 19,46 % respectively and increasing HDL 66,06 %, a diet of canning edamame also decreasing total cholesterol, LDL, triglyceride by 22,98 %, 6,18 % , 5,69 % respectively and increasing HDL 10,05 % .

Decreasing of total cholesterol, LDL, triglycerides and increasing of HDL probably because the role of edamame dietary fiber. This can be seen from total dietary fiber of edamame defatted & deproteinized have highest value, followed by edamame defatted, freezing edamame and canning edamame . Although in formulation of feed based on isofiber 5 %, but in each type of diet there were differences the constituent components of fiber especially water soluble fiber and water insoluble fibers.

Water soluble fiber in defatted&deproteinized edamame flour possibility increasing viscosity in intestines, so that decreasing the rate of absorption intestinal sterol (Holf *et al*. 1979). Masdar, (1983) showed that soybean fiber had high viscosity because it contains pectin, galaktomanan and arabinogalaktan. Other researchers also stated the viscous polysaccharides decreasing rate of absorption glucose and intestinal sterol (Anderson *et.al*. 2009, Chang *et al* 2008,

Wood *et.al,* 1990) and increasing viscosity in intestines and slower transit time and delaying empthying of the stomach (Anderson *et.al.* 1999, Slavin, 2013). Hence, decreasing cholesterol level in rats which were given defatted& deproteinized edamame feed might be due to ability of fiber in bile acid binding and excretion with feces. This state prompted the sinthesis of bile acids with the raw material of cholesterol and it decreased in cholesterol.

While the role of insoluble fiber of edamame flour to decrease the cholesterol would probably only little, although edamame flour dominated by insoluble fibers. Previous researchers reported that insoluble fibers have a few role in reduction of cholesterol. This is shown by Qureshi (2002) that decreasing of serum cholesterol, LDL and triglycerides with the intake of insoluble fiber from rice bran10 g for 8 weeks by diabetes patients type 1 and 2.

McIntosh (2001) added that a possible decreased of cholesterol by dietary fibers through inhibition of hepatic cholesterol biosynthesis because influence of propionic acid resulted from the fibers fermentation to inhibite of HMG-COA reductase activities.

Profile lipids can be used to predict the risks of atherosclerosis and coronary heart disease. Some researchers showed that AI (atherosclerosis index) and AIP (atherogenic index of plasma) is predictor to analyse the risk of coronary heart disease and atherosclerosis (Dobiasova, 2001, 2004). The value of AI on rats which diet of defatted &d eproteinized edamame diet decrease from 6,72 to

1,45 and for the defatted edamame diet from 6.25 to 2.11, whereas freezing edamame diet from 6.75 to 3,50 and canning edamame from 5,61 to 4,78.

Meanwhile, AIP value of rats fed defatted & deproteinized edamame diet showed a significant decrease after the diet intervention. The AIP of *defffated&deproteinized* flour diet is the lowest (0,29) among the other diet in this study. High efectivity in decreasing AIP of *defffated&deproteinized* flour was espected to decrease the risk of CHD.

4.4.3 Blood Glucose Levels

Decreasing in blood sugar levels in the group of rats fed with defatted edamame, defatted & deproteinized, freezing, canning were 44,81 % 56,68 % , 38,15 % and 29,45 %, respectively. Rats given the diet of defatted & deproteinized edamame diet showed the highest reduce of glucose levels than the other diet groups. The reduction of of glucose levels might be due to the influence of dietary fiber in defatted & deproteinized edamame diet, from 75.14 % insoluble fiber and 22,76 % soluble fiber, respectively. Soluble components probably have a higher roles in decreasing of glucose levels because of its viscous properties of the soluble fiber as sugested by other researchers (Jenkins *et al* .1978 in Rorie and Fahey, 2013) . Viscous soluble fiber to improve the viscosity of chyme, so that slows mixing chyme, slows interactions with enzymes digestion of nutrients, slows down breakdown of easy absorbed complex nutrients components and overall slows the absorption of glucose and other nutrients.

4.4.4. The Rats Digesta Properties

a. Weight of Digesta

No difference weight of rats digesta from all groups of diet, probably because of the similarities of feed intake levels of fermentation resulted in similarities in number of microbia and bacteria metabolite as well as undigested component in the caecum.

b. The water content of digesta

The edamame diets give digesta water content of rats (around 74,59 % - 85,49 %) higher than standard diet (66,68 %). It is possible because the influence of dietary fiber. Although number of dietary fiber used in isofiber diets formulation, but each type of diet have differences type of fiber so influential in digesta water content.

c. SCFA (*Short Chain Fatty Acid*)

The concentration of acetic acid, propionic acid , butyric acid and total SCFA of rats digesta which given freezing edamame feed and canning edamame feed show the real difference to other third diet and have highest value. This probably because of the composition of freezing and canning edamame flour contains FOS and raffinose used selectively to support the growth of probiotics microbes, so produces highest SCFA. Beside this, possibility of establishment RS or other unexamined components resulted in the processing can suppot the growth of microbes.

d. pH

The pH digesta of rats which were given diets of defatted, defatted& deproteinized and freezing edamame showing higher than pH of rats digesta given standard diet. There is a difference in pH value of this study, possibility because different components of fiber source constituent used in diet. It is results more dominant microbes differences to grow and provide difference in organic acids more dominant produced that affect pH value.

V. Conclusion

Based on results and discussion, this research can be concluded that removal of fat and protein increase total levels of carbohydrate, dietary fiber, decrease oligosaccharides in defatted edamame flour, and increase defatted & deproteinized edamame flour. Increasing in dietary fiber on products impact to the physicochemical properties (decreasing value of WHC and OHC). The physiological effects of defatted & deproteinized edamame flour is potential to improve the profile of lipid (hypolipidemic) and decreasing blood glucose levels (hypoglicemic), while freezing and canning edamame flour give more positive effect to maintain colon health.

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