The Potential Chito-Oligosaccharide (COS) as Natural Prebiotic and Preservatives on Synbiotic Tofu in Indonesia

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***Abstract*—Chito-oligosaccharide (COS) is a glycoprotein bond 1.4 glucosamine, are synthezied from chitin deacetylation result from waste of shrimp and crabs sheell there were potential and abundant in Indonesia. COS has a uniqueness that is polycationic proteins are able to protect and reduce the rate of growth of pathogenic bacteria. Tofu is one of the processed coagulation soybean products that contain proteins, fats, carbohydrates and fiber. The concept of food fortification can be used to characterize food biosuplemen health improvement as a functional food. The results show the potential of Chito-oligosaccharide 2% w/w was an integrated in the production of synbiotic tofu expected to be multifunctional as a prebiotic and a natural preservative can serve as a functional food.**

***Index Terms*—Chito-oligosaccharides, preservatives, prebiotic, synbiotic, tofu**

I. Introduction

Tofu is one of the processed soy products as functional food containing proteins, fats, carbohydrates and fiber; is made through the process of coagulation proteins that semi-solid form. Tofu as a non durable food products and the manufacturing process is generally carried out in the conventional or traditional in terms of equipment, methods and marketing. Therefore, durability of tofu only 1 day then it becomes a problem for society and tofu factory to be limited in accordance with the amount of sales or price that fluctuate on a daily. This is due to the tofu factory process that are traditional method and there has been no innovation in terms of production out of the equipment, methods, and marketable of product.

Chito-oligosaccharides (COS) derivatives of chitosan, a compound complex process results deacetylation of chitin which has 1.4 glucosamine bond and thus is able to function as an antimicrobial preservative. COS can be obtained from fishery waste materials such as shrimp head, shell crabs are abundant in Indonesia. The use of COS as a natural preservative in synbiotic tofu have not been popular in the Indonesian people, this is due to the knowledge and information about the COS as a natural preservative is still limited. COS has a uniqueness that is polycationic proteins are able to protect and reduce the rate of growth of pathogenic bacteria. COS as a potential material as "*alternative antibiotics*" has a value of more safety or harmless without causing residue. COS can be used as a natural prebiotic in yoghurt as functional food [1].

Synbiotic (probiotics and prebiotics) concept recently used for the characterization of foods and healthy prebiotic useful to improve the survival of probiotics in the digestive tract so that implantation is more efficient for microbiota colonization, with the effects of oligosaccharides in the stimulation growth activity between the two exogenous bacteria (probiotics) and endogenous [2].

Availability of raw materials abundant fishery waste in Indonesia for the synthesis of COS, the production process is relatively simple, the short time processing and simple equipment allow COS can be used as a natural preservative for the production of highly nutritious synbiotic tofu. Application the potential of an integrated COS as a natural prebiotic and preservative in production

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synbiotic tofu expected to provide value-added technological aspects, economic, social and ultimately may improve welfare. Synbiotic tofu to be expected can be used as a functional food for increased immunostimulatory.

II. Material and Methods

A. *Synthesis of Chito-oligosaccharide*

Chito-oligosaccharida was composed from waste of crab shell and shrimp shell by chitin deacetylation method.

B. *Production of Synbiotic Tofu*

In Indonesia, tofu production stages as follows soybeans are washed and soaked for 3 hours. Soybean drained, and put into the milling machine to become whey. Soy whey put in a frying pan containing boiling water, and allowed to boil for 15 minutes. Liquid slurry then filtered with a cloth and obtained is a solution soymilk. COS and probiotics (Lactic Acid Bacteria) was poured into a slurry of soy and allowed to form coagulant. Coagulant formed an pressed to synbiotic tofu.

C. *Analytical Methods*

Analysis of fermentation products include measurement of level protein with Lowry method, fat content with soxhletation method and microbiological methods (*Escherichia coli* and Salmonella) and organoleptic test with methods Hedonic test for the level of preference (color, smell, consistency, flavor) by panelists.

III. Results and Discusion

A. *Synthesis of Chito-oligosaccharide*

Chitosan can be synthesized from deacetylation of chitin chitosan as in Fig. 1 through the process and have a degree of deacetylation 80-90%. Chitosan is insoluble in water but soluble in acidic solvents with a pH below 6.0 [3]. Common solvent used to dissolve chitosan is 1% acetic acid at a pH of about 4.0. At pH above 7.0, the stability is very limited solubility of chitosan polyelectrolyte complexes formed with anionic hydrocolloid gel so it can be used as an emulsifier, chelating and coagulant [4], [5]. Chitosan showed antibacterial activity, antimetastatic, antisteoporotic, immunoadjuvant and *in vitro* biocompatibility of wound [6], [7]. The results showed that chitosan is able to absorb the fat that can reduce cholesterol [8].

B. *Processing of Synbiotic Tofu*

Yellow soy beans are used as a raw material for making tofu synbiotic therefore contains protein so it is ideal for diet food, low in saturated fat and cholesterol free, rich in minerals and vitamins, natural foods are healthy and free of toxic chemicals. As in Fig. 2, the processing of tofu sinbiotic consists of 3 stages: 1) extraction the soy protein using water solvent, 2) the process of denaturation proteins in the extract solution by lowering the pH of the solution, 3) pressing to separate and condense the protein from the whey. The process of submersion soybeans soaked in water for 8-12 hours, it makes soybeans will absorb water until it reaches a saturation limit and yield of soybean become soft thus simplifying the process of milling. Besides submersion, it can provide better dispersion the extraction of soybean, as well as reducing the typical odor of soybean [9], [10]. Milling aiming for soy protein extraction easier and the addition of water as much as 8-10 times the amount of processed soy. Soy milk extraction process is affected by temperature, extraction can be done with cold water or hot water (80-100^{o}C). During the extraction, the higher temperature and speed are results the greater the amount of material extracted . Therefore, the processing tofu, the material is extracted proteins that cause warming and denatured proteins are difficult to dissolve in water. Chito-oligosaccharides from fishery waste as a source of prebiotics. Additionally biopreparation synbiotic in yogurt provide a synergistic effect as lowering cholesterol levels in vitro and in vivo [11].

Figure 1. Synthesis of chitosan.

Figure 2. Processing of synbiotic tofu on laboratory scale.

C. Analysis of Product Synbiotic Tofu

Analysis of fermentation products include measurement of level protein with Lowry method, fat content with soxhletation method and microbiological methods (*Escherichia coli* and Salmonella) and organoleptic test with methods Hedonic test for the level of preference (color , smell , consistency , flavor) by panelists.

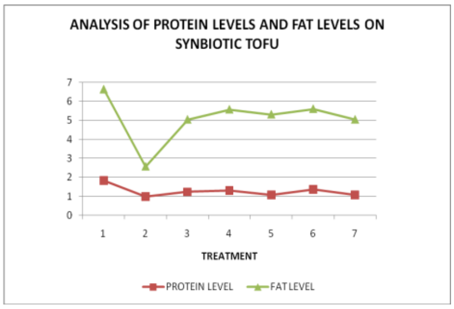

Figure 3. Graph analysis of protein levels and fat levels synbiotic tofu.

The results of chemical analysis includes protein level and fat level showed that treatment of lower protein level is compared with controls (see Table I). This is due to soy proteins undergo denaturation during the addition of acid. Tofu is a product processed denatured of soy protein. As in Fig. 3, the results of the analysis of the higher fat level out of control , this is due to the flavonoid soy beans or isoflavones and linoleic acid in soy bean are high level. Isoflavones in soy protein are dominated genistein and daidzein, whereas the protein predominantly daidzein and soy germ is glisitein [12]. Isoflavones that are known as an antioxidant and anticancer substance. Isoflavones have the potential unsaturated fatty acids that cannot be synthesized in the body and beneficial prevention of coronary heart disease.

TABLE I. ANALYSIS OF PROTEIN LEVEL AND FAT LEVEL ON SYNBIOTIC TOFU

Treatment	Protein level (%w/w)	Fat level (%w/w)	*E. coli*	Salmonella
1. Soy bean (control)	1.83	4.80	-	-
2. Rice bran	0.99	1.58	-	-
Soybean 3. + 2% chitosan shrimp	0.13	6.51	-	-
4. + 2% chitosan crab	0.18	7.94	-	-
5. + 2% chitosan market	0.14	6.71	-	-
6. + 2% FOS	0.13	6.81	-	-
7. + 2% Malto-dextrin	0.20	7.63	-	-

The results of organoleptic test showed that the addition of prebiotics: Chito-oliogsaccharide, rice bran, Fructo-oligosaccharide (FOS) and maltodextrin does not affect the color and odor but can improve the texture and taste of tofu. Chitosan (COS) can be used as a natural preservative in the know know that the shelf life is 7 days longer without organoleptics changes [13]. The analyzed of microbiological is indicated *Escherichia coli* and Salmonella on all sample are negative, therefore the product of synbiotic tofu are safe to consumed as a functional food.

TABLE II. ANALYSIS OF CHOLESTEROL LEVELS

	Treatment	Cholesterol level (mg/dL)
1.	Negative control (feed)	81.7
2.	Positive control (feed + fat)	185.3
	Feed + fat + bran + :	
3.	2% chitosan shrimp	103.3
4.	2% chitosan crab	114.0
5.	2% chitosan laboratory	97.3
6.	2% chitosan crab and probiotic	106.3
7.	2% FOS (Fructo-oligosacharide)	103.3
8.	2% Maltodextrin	122.3

Results of *in vivo* studies showed Chitooligosakarida can serve as a natural prebiotic antihypercholesterolemia (see Table II). The mechanism of COS as an anti-hypercholesterolemia associated Chitooligosakarida role in the metabolism of carbohydrates and bioadsorbent very effective especially as microparticles with high porosity properties so as to bind macromolecular compounds such as fats. Addition of probiotic LAB (Lactic Acid Bacteria) are expected to provide a synergistic effect with Chitooligosakarida as a natural prebiotic derived from crab shell waste.

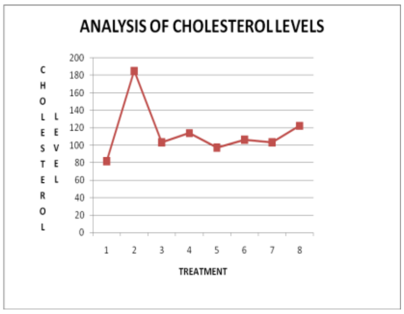

Figure 4. Graph analysis of cholesterol levels *in vivo*.

The results of *in vitro* experiments showed as Fig. 4. The addition of COS is able to significantly enhance the growth of probiotic *Lactobacillus* strains were able to assimilate and cholesterol. Mechanism as probiotics (Lactic Acid Bacteria) in lowering serum cholesterol levels associated with the ability to produce bile salt dehydrogenase enzymes that play a role in cholesterol metabolism. Assimilation of cholesterol associated with the presence of bile salts and cholesterol removal from the medium increases the concentration of bile salts. However, strains of *L. Actobacillus acidophilus* did not show the greatest ability to assimilate cholesterol; strains that can tolerate bile salts (bile tolerance) the actual minimum assimilate cholesterol. Assimilation of cholesterol by *L. Acidophilus* during refrigerated in non-fermented milk showed that uptake of cholesterol associated with the growth and viability of bacteria [14]-[17]. Several types of prebiotics are able to use lactosucrose, soybean oligosacharida, palatinose, isomaltive oligosaccharida, glucooligosaccharida, xylooligosaccharida, lactulose, lactitol and xylitol, sorbitol and mannitol. Several groups including prebiotic substrate is starch (cellulose, hemicellulose, lignin) are not soluble in water, fiber, oligosaccharides and sugar alcohols. The probiotics and prebiotics Maltodextrin 2%, 2% Fruktooligosaccharide and a mixture of both (1:1) were able to suppress the growth of pathogenic *Escherichia coli* [18], [19]. The application of COS as a natural prebiotic and preservative on synbiotic tofu can serve as a functional food [20], [21].

IV. Conclusion

The potential of Chito-oligosaccharide 2% w/w could be integrated as a prebiotic and a natural preservative in synbiotic tofu. Synbiotic tofu to be expected can be used as a functional food for increased immunostimulatory.

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