International Journal of Public Health Excellence (IJPHE)
Vol. 3, No. 1, December 2023, pp. 288~299
Journal Homepage: https://ejournal.ipinternasional.com/index.php/ijphe
ISSN: 2809-9826, DOI: 10.55299/ijphe.v3i1.629

Synbiotic Soygurt Tempe Extract as A Functional Drink for Stunting Prevention

Mellia Silvy Irdianty[1], Agnes Sri Harti[2], Erlina Windyastuti[3]
[1,2,3] Department of Nursing, Faculty of Health Sciences, Kusuma Husada University of Surakarta, Surakarta, Indonesia

Article Info

Article history:

Received October 25, 2023
Revised November 02, 2023
Accepted December 31, 2023

Corresponding Author:

Mellia Silvy Irdianty
Department of Nursing,
Faculty of Health Sciences,
Kusuma Husada University of Surakarta, Surakarta,
Indonesia
Email:
silvy.irdianty@gmail.com

ABSTRACT

Stunting is a chronic malnutrition problem caused by insufficient nutritional intake for a long time due to the provision of food that does not meet nutritional needs. Toddlers with stunting are a chronic nutritional problem which can be caused by many factors, socioeconomic conditions, maternal nutrition during pregnancy, illness in infants, and lack of nutritional intake in infants. Synbiotics (prebiotics and probiotics) as an effective alternative for stunting Analyzing the effectiveness of prebiotic tempeh soyghurt as a functional drink for stunting prevention. The research method is an experimental research including fermenting prebiotic tempeh and prebiotic tempe extract, making prebiotic tempeh soygurt, and analyzing the nutritional content and product quality of probiotic tempeh soygurt referring to SNI 7552:2009 concerning flavored fermented milk including chemical physics analysis, namely the hedonic method organoleptic test, pH, acidity, protein, fat, ash content, fiber, water content and microbiological tests, namely MPN coliform and Salmonella sp. Data analysis used bivariate and multivariate Anova tests. Organoleptic test results for prebiotic soygurt extract of tempeh product with the addition of chitosan (0.5%; 1.0%; 1.5%; 2.0%) using lactic acid bacteria (LAB) inoculum in the rather dislike category (value 3.00) to rather like (value 4.50) . Physical test results showed liquid texture, yellowish color, aroma of tempeh extract, sour taste. Chemical test results (w/b) fat content 3.34%; milk solids 13.19%, non-fat milk solids 9.25%; proteins 0.69%; ash content 0.19%, acidity 0.69% and sugar content 0.59%. Microbiological tests showed MPN coliform <3 cells/100 ml sample and Salmonella sp/25 ml negative results.
CONCLUSION: Soygurt extract tempe prebiotic product meets the physical, chemical and microbiological requirements so that it can function as a functional drink for stunting prevention can be concluded that the accuracy of measuring body weight depends on gender.

Keywords: *Extract, Prebiotic Tempeh, Functional Drink, Soygurt, LAB*

1. INTRODUCTION

Stunting is a condition of the nutritional status of toddlers who have a length or height that is classified as less when compared to age. Toddlers with stunting include chronic nutritional problems which can be caused by many factors, socioeconomic conditions, maternal nutrition during pregnancy, illness in infants, and lack of nutritional intake in infants [13]. Based on data from the 2021 Indonesian Toddler Nutrition Status Survey (SSGBI), the prevalence of stunting is currently still at 24.4 percent or 5.33 million toddlers [14]. Even though the percentage has decreased from the previous year, the incidence of stunting is still relatively high. The Indonesian government has targeted the Stunting Reduction Program to 14% in 2024 by making maximum efforts at Integrated Service Posts [13]-[14].

In Indonesia, children tend to prefer snacks compared to balanced meals because they contain lots of fat (59.6%) and calories (40%) but contain little protein and micronutrients. The results of Mulyaningsih's latest research (2021) state that there is a positive relationship

between the high frequency of snack consumption and the risk of child stunting. Protein and micronutrients are important nutrients that can affect bone formation (calcium), bone length development (zinc) and increase in intrauterine femur length (supplements), so an innovative functional food is needed that is liked by children but has a high nutritional content of protein [33] .

Soy milk is a product like cow's milk but made from soy extracts. Soy milk protein has an amino acid composition similar to cow's milk, so it is very good as a substitute for cow's milk, especially for those who are allergic to lactose intolerance or for those who don't like cow's milk or have less purchasing power. In addition to its good protein quality, soybeans are also easy to obtain, contain high levels of essential unsaturated fatty acids (linoleic) and do not contain cholesterol, so regular consumption of soybeans can reduce degenerative diseases. The nutritional value of soy milk is no less than cow's milk. Liquid soy milk contains as much as 3.5 grams of protein, while cow's milk only contains 3.2 grams per 100 grams. The quality of soy milk protein in the form of a single food is 80% of the quality of cow's milk protein [6].

Yogurt on the market generally uses animal milk, namely cow's milk, while yogurt made from vegetable protein, especially soybeans, is not widely known. Soyghurt is a way of fortifying fermented yogurt based on vegetable protein, which is made from soybean seeds [1]. Based on survey results through web applications, soyghurt that is known and available on the market is generally based on soybean seed extract without the addition of prebiotics and is fermented with probiotics or LAB, including Lactobacillus acidophilus, L. bulgaricus, Streptococcus thermophilus. Optimization of probiotics can be done by selecting or selecting the right strain, monitoring the fermentation process and its application in the right prebiotic food. Therefore the concept of synbiotics in functional food is an alternative to be developed in soy milk or soyghurt fermented foods so that they can be used to prevent stunting [2].

The concept of synbiotics (a mixture of probiotics and prebiotics) has recently been used for the characterization of health promoting foods and supplements used as important dietary constituents in humans. Synbiotic biosupplements applied in soy milk yogurt are expected to provide an immunostimulating effect and reduce cholesterol levels as a synbiotic food supplement that is multipurpose, safe and economical. Synbiotic biopreparations in yogurt provide a synergistic effect in reducing cholesterol levels in vitro and in vivo. Rice bran and Chitooligosarida as derivatives of chitosan as natural prebiotics in synbiotic prebiotic tempeh and tofu showed significant antihyperglycemic and hypercholesterolemic results [7] & [9]. The addition of prebiotics (bran and chitosan) to prebiotic tempeh as a raw material for yogurt is expected to produce synbiotic soyghurt products that not only have the ability to maintain digestive health, but also contain antioxidants as free anti-radicals given the current changes in people's consumption patterns.

Soyghurt tempe prebiotic is a krenova (creative and innovative) food product because it has a novelty and uniqueness using prebiotic tempe extract as raw material. Prebiotic Tempe which has obtained Patent Certificate No. ID P000050238 can be used as a raw material for making prebiotic tempeh soyghurt so that it is beneficial from health, technological, economic and social aspects so that it can function as healthy and safety food, functional food, fun for diet and one for all.

The conceptual concept of this research is the development of a patented prototype of prebiotic tempeh into synbiotic tempeh soygurt as a Krenova product through a probiotic LAB fermentation process based on raw materials of prebiotic tempeh extract (bran and chitosan) so that it can function as a functional drink based on science and technology, halal and economical for stunting prevention. The essence and usefulness of the results of this research are related to the scientific competence of the research team in the field of nursing care for children and their participation in supporting the success of the Government's program in tackling and reducing

the incidence of stunting in Indonesia. The aim of the study was to analyze the effectiveness of prebiotic soyghurt tempeh as a drink

2. METHODS

This type of research is experimental research. This study uses the True Experimental design. The research was conducted at the Nutrition and Biochemistry Laboratory, Kusuma Husada University, Surakarta and the Microbiology and Pharmacology Laboratory, Setia Budi University, Surakarta. Implementation time in December 2022 – January 2023.

The tools used are incubator, autoclave, centrifuge, filter membrane, oven, analytical balance, pH meter, oven, UV-Vis spectrophotometer, micropipette, petri dish, test tube, vortex, micropipette, appendorf.

The main ingredients in this study were soybeans, rice bran and chitosan. Soybeans are obtained from the place of manufacture of the tempe industry / UKM in the Krajan Surakarta area. Rice bran was obtained from the Surakarta Legi Market. Food grade chitosan was obtained from PT. Biotechsurindo Cirebon. Yogurt starter used from yogurt or fermented milk products on the market, namely Inoculum A (Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, and Bifidobacterium sp); Inoculum B (Lactobacillus delbrueckii subsp bulgaricus and Streptococcus thermophilus); Inoculum C (Lactobacillus casei Shirota strain).

The stages of the research included the preparation of prebiotic tempeh soyghurt and analysis of physicochemical and microbiological tests as well as in vivo tests.

1. Preparation of Prebiotic Soyghurt Tempe

Prebiotic Tempeh Fermentation

The method of making soybean tempeh is based on the method of making Kasmidjo (1990) with research modifications, namely by soaking twice and boiling twice. Soybeans are soaked in water for two hours and boiled for 30 minutes. Soybeans are soaked again for 24 hours and the skin is removed. Soybeans were boiled again for 30 minutes then drained and air dried before inoculating (Widowati et al, 2011). For the manufacture of prebiotic tempeh, it is prepared by mixing soybean seeds with rice bran which has been steamed for 15 minutes at a ratio of 10:1 with the addition of chitosan formulation, namely: 0.5%; 1.0%; 1.5%, 2.0% v/w according to treatment and inoculated with 2% (w/w) tempeh yeast. Furthermore, soybean seeds with chitosan bran are wrapped in banana leaves and fermented for 48 hours.

Yoghurt Starter / Inoculum

Yogurt starter isolated from commercial yogurt (inoculum A, B, C) was inoculated 25 ml aseptically into 50 ml of tempeh milk and then fermented for 8 hours at 40oC. The main starter is inoculated with 5% in tempeh extract which will be made into yogurt [10].

Sinbiotic Tempe Soyghurt Fermentation

Tempeh that has been compacted as much as 250 grams is cut into 1 cm2 cubes and then boiled for 5 minutes. The purpose of boiling is to kill Rhizopus sp. on tempeh. Tempeh is blended and warm water is added with a ratio of 1:3 so that it becomes tempeh porridge. Tempe porridge is filtered using a filter cloth. The result of filtering tempeh porridge is tempeh milk. Tempe milk is added with skim milk as much as 15% (w/v) then pasteurized for 15-30 minutes at 70-80oC. Tempe milk is allowed to stand until the temperature is 40oC which is the optimal temperature for the growth of probiotic bacteria. Milk was then inoculated aseptically with 5% starter and incubated at 400C for 12 hours.

2. Analysis of Nutritional Content and Quality Test of Prebiotic Soygurt Tempe Products as Functional Drinks

Purpose: to analyze the potency and nutritional content of prebiotic soyghurt tempeh in physico-chemical and microbiological terms. Analysis of nutritional content and quality test of prebiotic tempeh soygurt refers to SNI 7552:2009 concerning flavored fermented milk.

Types of analysis include:

a. Testing the organoleptic properties (color, smell, consistency, taste) uses the Hedonic method to determine the level of consumer preference
b. Chemical analysis includes fat, non-fat milk solids, protein, ash, titrated acidity, metal contamination (Pb, Hg, As)
c. Microbiological analysis including coliform
d. Microbiological analysis of prebiotic tempeh soygurt including coliform and identification of Salmonella sp. Coliform analysis used the MPN (Most Probable Number) method while the identification of Salmonella sp was carried out on selective Bismuth Sulfite Agar media.

3. In Vivo Prebiotic Soyghurt Tempe Effectiveness Test for Stunting Prevention

Testing the effectiveness of prebiotic soyghurt tempeh in vivo for stunting prevention was carried out in 2 groups of mice, namely the control group and the treatment group. Each group consisted of 5 mice test animals. Mice adaptation was carried out for 3 days before being given treatment. The control group was given feed without treatment while the treatment group was given prebiotic soyghurt tempeh ad libitum at a dose of 1 x per day. The treatment was carried out for 2 weeks. The parameter measurements were based on weighing the pre and post rats after treatment every 3 days during the treatment. The dependent variable was the dose of prebiotic soyghurt tempeh according to the dose. The independent variable is the body weight of each rat

Data analysis

Data analysis technique performed is using statistical data analysis techniques. In this study, the mice's body weight was measured before being given prebiotic yogurt and after being given prebiotic yogurt. The resulting data is in the form of ratio data, namely the weight of mice. Analysis and analysis with T-test

3. RESULTS AND DISCUSSION

The results of the soygurt product with prebiotic tempe extract are liquid consistency of yellowish color, distinctive aroma of tempe extract and sour taste so that it can be categorized as a flavored fermented milk beverage product. Product quality test chemically and microbiologically refers to SNI 7552:2009 concerning flavored fermented milk.

SNI 01-2981-2009 states that the criteria for yogurt taste are normal or typical sour. Various acids and aromas are the result of different fermentation processes. Fermented milk that has not too high acidity will be preferred by the public so that it can increase milk consumption [9] & [11]. The taste of food is one of the important parameters that influence consumer acceptance of a food product. The taste produced is influenced by the components that are in the ingredients and the process they go through. Taste is a very determining factor in the consumer's final decision to refuse or accept a food, even though the other evaluation parameters are better, if the taste of the food is not liked then the product will be rejected.

Testing the organoleptic properties of prebiotic tempeh soygurt (color, smell, consistency, taste) using the Hedonic method

Organoleptic tests were carried out on 3 treatment groups of 3 types of inoculum A, B, and C using the hedonic (preference) method with parameters tested including acidity, taste, and aroma carried out by 30 panelists.

Table 1. Results of organoleptic and pH tests with inoculums A, B, C

No.	Parameter	Type of inoculum			Sig
		A	B	C	
1	Rasa	3.00 – 4.33	3.00 – 4.50	3.00 – 4.75	0.224
2	Tekstur	3.33 – 4.00	3.25 – 4.00	3.00 – 4.00	0.732
3	Aroma	3.00 – 4.00	3.25 – 4.00	3.00 – 4.50	0.576
4	pH	4.04 – 4.15	3.96 – 4.16	3.76 – 4.18	

The results of the study in terms of the taste of inoculum types A, B, and C were not significantly different from the category values rather dislike (3.00) to rather like (4.50) but have not yet reached the liking stage (value 5). This is due to the respondents comparing it with actual yogurt products or on the market yogurt products based on full cream milk, which have a semi-solid consistency, sweet and sour taste. While the organoleptic product of soygurt extract of prebiotic tempeh is a non-homogeneous liquid texture because the tempe extract and skim milk are not evenly mixed; scented with tempeh and langu extract from soybean seed extract and tends to be high in acidity without a sweet taste. This is due to the absence of added sugar or sucrose and lactose in prebiotic tempeh extract soygurt products as carbohydrates in animal milk.

Based on this, the selection of the type of inoculum greatly influences the product organoleptic related to the taste, aroma and texture of the resulting prebiotic soybean extract soybean product. The type of inoculum is related to the type of LAB microorganism (Lactic Acid Bacteria) as the actor in the lactic acid fermentation process carried out by LAB. In general, lactic acid bacteria originating from the human digestive tract such as Lactobacillus acidophilus, L. casei Shirota, Bifidobacterium bifidum, L. gasseri, and L. reuteri can act as good probiotics. Meanwhile, Streptococcus thermophilus, L. bulgaricus, L. lactis, L. cremoris, and L. diacetylactis are fermented cultures of dairy products that cannot reach the human intestine alive [5].

The fermentation process by LAB is because LAB has a lactic acid fermentation pathway, namely homo-fermentation and/or hetero-fermentation which can affect the end product of the fermentation results, namely acidity, total protein, pH, and fat. Soy milk has a nutritional content almost the same as cow's milk because the protein content is 3.5 – 4.0%, but does not contain casein so that soy milk can be used as a substitute for cow's milk which is fermented by BAL so it is known as soygurt. There is no casein in soy milk, this causes the consistency of tempe extract soygurt which is liquid. The formation of a semi-solid texture due to the formation of Calcium lactate in cow's milk yogurt occurs due to the reaction between casein and lactic acid resulting from the fermentation of carbohydrate lactose in cow's milk. The types of carbohydrates in soy milk are different from cow's milk, especially glucose and lactose. LAB is classified as lactic acid bacteria because it is able to ferment lactose through the lactic acid fermentation pathway, namely homo-fermentation or hetero-fermentation [3], [6].

The end result of the fermentation process carried out by the LAB type greatly affects the acidity or pH level of the product. The more accumulation of lactic acid formation produced by LAB fermentation, the lower the pH which has an impact on the increasingly acidic organoleptic product. The level of acidity that is too high is not liked by respondents, because respondents tend to like fermented yogurt products with a slightly sour and sweet taste so that fermented milk drinks are made with the addition of sucrose as an additional ingredient and

essence or flavorings such as fruit aromatic compounds to increase organoleptic value. The unpleasant smell of soygurt prebiotic tempe extract can be overcome by adding additional ingredients flavored with strawberries, melon, grapes, orange, peach and coloring according to the flavors used to attract consumers.

The texture includes the consistency and color of the soygurt product, prebiotic tempe extract, which is a yellowish color liquid produced from prebiotic tempe extract, namely light brown color, the color of soybean and Rhizopus sp mushroom extracts, and the addition of 15% skim milk, which makes the final color yellowish due to the effect of the addition skim milk Skim milk is not soluble in prebiotic tempe extract, this causes the prebiotic tempe extract soygurt product as a fermented drink in liquid form, yellow in color. The results of the analysis test showed that the color of the inoculum A, B, and C groups had no effect. The color of the prebiotic tempe extract yogurt product gives a distinctive color because respondents generally know that yogurt products are white in color because they are based on the color of cow's milk, while this gives the prebiotic tempe extract a distinctive color. Meanwhile, the liquid consistency is not semi-solid like yogurt products from cow's milk because in soyghurt, prebiotic tempe extract, there is the addition of 15% skim milk without the addition of animal milk related to the casein content. The formation of a semi-solid consistency due to the reaction of calcium lactate. In soyhurt, the prebiotic tempe extract does not contain calcium, so the consistency remains runny and the skim milk is suspended in the tempe extract. Denaturation can change the properties of proteins to make them insoluble and more viscous [25]. This clumping can be caused by heating, giving acid, adding enzymes, mechanical treatment and the presence of heavy metals. In terms of texture (color and consistency) the results of the preference test in the texture category are from a rating of somewhat dislike (value 3) to rather like (value 4).

Based on the results of organoleptic tests related to taste, aroma and texture of the soygurt product with prebiotic tempe extract with the results of liquid consistency, yellowish color, distinctive aroma of tempe extract and sour taste so that it can be categorized as a flavored fermented milk beverage product. Product quality test chemically and microbiologically refers to SNI 7552:2009 concerning flavored fermented milk.

Chemical analysis of prebiotic tempeh soygurt for pH was carried out at the Biochemistry Laboratory, Kusuma Husada University, Surakarta using a pH meter with inoculums A, B, C showing a pH range of 3.76 – 4.13, while the pH in the negative control group of non-prebiotic tempe extract with inoculum C showed a pH 3.79, while the pH of the positive control product showed a pH of 4.40. This shows that the pH of the soygurt product, prebiotic tempe extract and chitosan allows the lactic acid fermentation process to be carried out by LAB contained in the inoculum. The pH value after 48 hours of fermentation was lower than the pH of tempeh extract, which was 6.27 and the initial fermentation after the addition of chitosan was 5.50. The fermentation process carried out by L. plantarum caused the pH to drop. In homofermentative bacteria (L. plantarum) the existing carbohydrates are broken down into pyruvic acid via the EMP pathway which is then reduced by NADH 2 to become lactic acid. Soygurt inoculated with L. plantarum has a sour aroma, because most of the results of carbohydrate metabolism are lactic acid. This shows that in the fermentation process lactic acid is produced by lactic acid bacteria which can reduce the pH value [5].

Chemical analysis of prebiotic tempeh soygurt apart from pH analysis, other chemical parameters, namely analysis of acidity, protein, fat, antioxidants, ash content, fiber, moisture content was carried out in the laboratory of the Surakarta Goods Quality Testing and Certification Center (BPSMB) due to limited or no test equipment in full at the University of Kusuma Huada Surakarta. The chemical quality test of prebiotic tempeh soygurt for acidity, protein, fat, antioxidants, ash content, fiber, moisture content was carried out based on the

results of the organoleptic test that respondents preferred using inoculum C so that it was carried out on 2 samples of the CK and KCK groups as listed in table 2.

Table 2. Test results for acidity, protein, fat, antioxidants, ash content, fiber, water content in prebiotic tempeh soygurt.

No	Type of test	Content % (b/b)	
		CK1	KCK1
1	Lemak	3.34	1.56
2	Padatan susu	13.19	13.57
3	Padatan susu bukan lemak	9.25	7.86
4	Protein	0.69	0.69
5	Kadar abu	0.19	0.15
6	Keasaman	0.69	0.42
7	Kadar gula	0.59	4.15

Test reference no 1-6 : SNI 7552:2009; no. 7 : SNI 01-2892-1992
CK1 : prebiotic tempe extract + chitosan 1.0%; KCK1 = tempe extract + chitosan 1%

Fat content was calculated by the Soxlet method and expressed as % fat. The % fat content (w/w) in the prebiotic tempe extract sample with inoculum C (CK1) was 3.34 or higher than the tempe extract sample (KCK1) of 1.56%. The main fatty acid components of soybean triglycerides are unsaturated fatty acids which are dominated by linoleic acid, linolenic acid and a little oleic acid. These fatty acids are free of cholesterol and contain tocopherols, sterols and phospholipids such as lecithin and lipositol. About 60-75% of the fatty acids that make up cow's milk fat are saturated fatty acids, 23-30% unsaturated fatty acids and 4% polyunsaturated fatty acids. Saturated fats have longer chains than unsaturated fats, this makes the degradation of saturated fats more difficult and longer than unsaturated fats. The results showed that the fat content of yogurt decreased after 96 hours of fermentation because Lactobacillus was able to reduce fat content by being absorbed as a source of energy for growth. The use of L. casei and L. acidophilus inoculums with a concentration of 2% in yoghurt fermentation can increase the viscosity caused by protein denaturation by the lactic acid produced. Changes in viscosity are directly related to changes in the acidity of the resulting product [17].

The level of acidity is measured as a product of lactic acid which is related to the pH of the product. The end product of fermentation can affect the growth of LAB because each microbe has a minimum, maximum and optimum pH activity for growth. This is known as feed back inhibition growth. Lactic acid for each bacterium is influenced by the number of viable bacterial cells which can be shown by previous analysis that a high number of viable bacterial cells will produce a high lactic acid as well. Lactic acid is obtained by decomposing sugars in the form of glucose, lactose, sucrose, raffinose and stachyose fermentation media through the process of glycolysis [30]-[31].

Protein levels expressed in percent were measured using the Lowry method with Bovine Serum Albumin (BSA) as a standard solution. Protein content measured using the Lowry method aims to calculate the amount of protein dissolved in water. The results of analysis of protein content % (w/w) CK1 and KCK1 were the same 0.69. Protein levels are affected by the number of viable bacterial cells, an increase in the number of viable bacterial cells will increase the number of enzymes used to break down proteins (proteolytic activity) and increase protein synthesis, including protein-breaking enzymes (proteases) [32].

Microbiological analysis of prebiotic tempeh soygurt

Microbiological analysis of prebiotic tempeh soygurt refers to SNI 7552:2009 concerning flavored fermented milk, including coliform and identification of Salmonella sp.

Microbiological quality testing of prebiotic tempeh soygurt was carried out at the Microbiology Laboratory of Setia Budi University, Surakarta, due to the unavailability of media for coliform analysis and identification of Salmonella sp with the results as listed in table 3.

Table 3. Results of prebiotic tempeh soygurt microbiological testing.

Code product	Microbial Contamination Test	**Result**	Quality Requirements *	**Method**
CK 2	• Coliform Bacteria (MPN/ml) • *Salmonella sp*/ 25 ml	• < 3 sel / 100 ml sampel • Negative	• Max 10 • Negative	• MPN coliform • Test on Bismth Sulfite Agar selective media
KCK 2	• Coliform Bacteria (MPN/ml) • *Salmonella sp*/ 25 ml	• < 3 sel / 100 ml sampel • Negative	• Max 10 • Negative	• MPN coliform • Test on Bismth Sulfite Agar selective media

*according to SNI 7552:2009

Based on table 6, the results of microbiological testing of prebiotic soygurt tempeh showed that the product met the microbiological quality test requirements, namely MPN coliform <3 cells per 100 ml and the absence of Salmonella sp bacteria per 25 ml. This shows that the prebiotic tempeh soygurt product is safe when consumed as a functional drink because there are no coliform bacteria and Salmonella sp. Coliform bacteria are normal flora bacteria in human digestion and Salmonella sp especially Salmonella typhi is a pathogenic bacteria that causes typhoid fever infection. Testing food products from a microbiological point of view needs to be carried out to describe the quality or quality of raw materials, the manufacturing process and product packaging as indicators of healthy, hygienic and safe food products when consumed by consumers.

In Vivo Prebiotic Soygurt Tempe Effectiveness Test For Stunting Prevention

Testing the effectiveness of prebiotic tempeh soygurt in vivo for stunting prevention was carried out in 4 groups of mice, namely

KN = negative control group (no treatment)

KP = positive control group (by giving Yakult fermented milk)

K1 = treatment group (CK2) prebiotic tempe extract + chitosan 1.0%

K2 = treatment group (KCK2) tempe extract + chitosan 1.0%

Giving prebiotic tempeh soygurt ad libitum at a dose of 0.2 ml per body weight and given 1 x per day. The treatment was carried out for 2 weeks. The parameter measurements were based on the weight of the rats pre and post after treatment. Weighing is done every 3 days. The results of the in vivo prebiotic tempeh soygurt effectiveness test are as listed in table 4.

No. Treatment Code Mean body weight / gram of Sig

Pre (0) Post (12)

1 Negative control KN 21.6567 24.0867 .044

2 Positive control KP 20.9800 26.9467 .023

3 K1 CK2 22.6700 30.8600 .018

4 K2 KCK2 23.2467 31.7767 .007

Table 4. Results of testing the effectiveness of prebiotic tempeh soygurt in vivo

No.	Treatment	Code	Mean body weight / gram		Sig
			Pre (0)	Post (12)	
1	Negative control	KN	21.6567	24.0867	.044
2	Positive control	KP	20.9800	26.9467	.023
3	K1	CK2	22.6700	30.8600	.018
4	K2	KCK2	23.2467	31.7767	.007

Based on the multivariate test results, it was shown that the administration of prebiotic tempe extract and chitosan (CK2) or tempe and chitosan extract (KCK2) had a significant effect on the increase in body weight of the test animals. This shows that prebiotic tempe extract and chitosan have the potential as synbiotic drinks. Prebiotics are foods that cannot be digested by the body but are beneficial by selectively stimulating the growth of the activity of a number of colonic bacteria, namely the LAB group, thus improving health. Meanwhile, probiotics are living organisms that are consumed by the body in sufficient quantities to provide health benefits. A combination of prebiotics and probiotics, which is generally carried out through a fermentation process, is referred to as a synbiotic [27].

Synbiotic drinks include functional food, namely food (food and or drink) which contains one or more functional components and can be consumed daily, having multiple benefits for health. The benefits of functional food include being able to improve the normal flora of the intestine because it contains probiotics or LAB and prebiotics, namely fiber or oligosaccharides which can be used to increase prebiotic growth and improve the immune system because LAB produces bacteriocin compounds which can act as bioimmunostimulators. Synbiotic food includes functional food, namely there are probiotics and prebiotics so that they are antihypercholesterolemic and antihyperglycemic as well as anti-microbial (bacteriocin) so that they function to maintain digestive tract metabolism, including stunting prevention. Soygurt tempeh extract is prebiotic and ktiosan as synbiotic food because it contains probiotics, namely LAB inoculum and prebiotics, namely chitosan and soybean extract oligosaccharides which can increase the amount of LAB probiotics. Supplementation of probiotics or BAL in the lactic acid fermentation process can trigger the formation of short chain fatty acids (ALRP) or SCFA (Short Chain Fatty Acids), namely organic fatty acids consisting of 1 to 6 carbon atoms composed of the main components of acetic acid, propionic acid and butyric acid. (Puspitasari, 2016), which is able to increase the expression of precursor genes from GLP-1 through activation of free fatty acid receptors so that it plays a role in the metabolic prevention of type 2 DM [18]-[19], [8].

The type of ALRP from fermented soybean extract is a class of stachyose and raffinose oligosaccharides, which are able to function as a prebiotic, namely a class of fibers that cannot be digested by the human intestine but can be used or digested due to the activity of α-galactosidase, β-glucosidase, and β-fructosidase (invertase)) produced by LAB thereby increasing the amount of intestinal probiotics [23]. The results showed that ALRP helps the existence of normal intestinal flora to function optimally so that it can prevent the growth of tumor cells, encouraging the destruction of cancer cells in the colon [14]. Apart from the oligosaccharide group, a source of glucose in soybeans can also be obtained by hydrolyzing isoflavone glucoside compounds by the β-glucosidase enzyme to obtain glucose. The β-glucosidase enzyme plays a role in the hydrolysis of glucoside compounds by breaking the β-D-glucoside bonds to produce sugar compounds (glycones) and not sugars (aglycones) [28]. The bacterium L. plantarum B1765 had the highest activity of the β-glucosidase enzyme at the 18th hour in hydrolyzing isoflavone glucoside compounds in soybean juice (Huda et al, 2016). In addition, Lactobacillus plantarum SMN 025 showed β-glucosidase activity during soybean

fermentation, which was indicated by a decrease in the highest concentration of isoflavone glucosides at the 24th hour of fermentation [11].

The effect of probiotic yogurt consumption on inflammatory biomarkers in type 2 diabetes patients who consumed yogurt for 8 weeks could reduce HbA1C and TNF-a numbers. This means that consumption of yogurt can be a method of treatment for patients with complications of diabetes [24] [32] Other researchers say that consuming yogurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 for 4 weeks has a beneficial effect on blood glucose levels, glucosidated hemoglobin, diastolic blood pressure and blood cholesterol levels [20]. Lactobacillus can act as a cholesterol-lowering probiotic bacteria because the compounds produced by microbes compete with HMG CoA to bind to the HMG CoA reductase enzyme. The Lactobacillus genus as a probiotic has advantages in producing acids, bioactive compounds, and anti-microbial substances [4].

Stunting is still a health problem in Indonesia. One of the causes of this condition of failure to thrive is a lack of intake of nutrients (malnutrition). Prevention of stunting is usually focused on improving nutrition, especially intake of micronutrients. A number of studies report that micronutrient interventions cannot prevent stunting. Intestinal microbiota plays an important role in absorbing nutrients and accelerating the improvement of nutritional status to support the growth and development of stunted children. Modulation of the diversity of the composition of the gut microbiota is now one of the treatment targets to overcome dysbiosis that causes infections by inhibiting the growth of pathogenic bacteria, increasing the immune response and gastrointestinal health so that there is an increase in absorption of nutrients [22].

Synbiotics (prebiotics and probiotics) have a significant positive effect on gut microbiota which can induce SCFA, reduce dysbiosis and increase intake and nutritional status of stunted children. The results showed that consumption of sinbiotic powder for 90 days on changes in gut microbiota profile (Lactobacillus plantarum, Bifidobacterium and Enterobacteria), fecal characteristics, short-chain fatty acids, nutrient intake and nutritional status in stunted toddlers at the Yogyakarta Nutrition Recovery House [29]. Supplementation with synbiotic powder is expected to modulate the diversity of gut microbiota composition to overcome dysbiosis that occurs in stunted children. Sinbiotic powder can assist in the production of metabolites that are beneficial for the health of the digestive tract, namely Short Chain Fatty Acid (SCFA) so that it can reduce the incidence of stunting in Indonesia.

4. CONCLUSIONS

Organoleptic test results for soygurt product of prebiotic tempe extract with the addition of chitosan (0.5%; 1.0%; 1.5%; 2.0%) using BAL inoculum in the slightly dislike category (value 3.00) to rather like (value 4.50). Physical test results showed liquid texture, yellowish color, aroma of tempeh extract, sour taste. Chemical test results (w/b) fat content 3.34%; milk solids 13.19%, non-fat milk solids 9.25%; proteins 0.69%; ash content 0.19%, acidity 0.69% and sugar content 0.59%. Microbiological tests showed MPN coliform <3 cells/100 ml sample and Salmonella sp/25 ml negative results.

ACKNOWLEDGMENTS

Acknowledgments are extended to AIPVIKI which has facilitated through the 2022 Applied Nursing Research research scheme.

REFERENCES

[1] Anggraini, AA, M Devi, N Nurjanah, and N A Sunaryo, 2021. Chemical properties analysis of soygurt with ginger (Zingiber officinale var. roscoe) extract as functional foods. International Conference on Green Agro-industry and Bioeconomy. IOP Conf. Series: Earth and

Environmental Science 733 (2021) 012074. IOP Publishing. doi:10.1088/1755-1315/733/1/012074.
[2] Beckman, et al. 2021. Prevalence of Stunting and Relationship between Stunting and Associated Risk Factors With Academic Achievement and Cognitive Functions: A Cross_Sectional Study With South African Primary School Children. International Journal of Environment Research and Public Health, 18.4218.
[3] Choi H.J., Ahn J., Kim N.C., Kwak H.S., 2006. The effects of microencapsulated chitooligosaccharide on the physical and sensory properties of the milk. Asian-Australian journal of animal sciences. Volume. 19, No. 9, pages 1347-1353.
[4] Dewi, IP, Septriani R., Verawaty, Mulyani, D., 2019. Antihyperglycemic Power of Yogurt in Male White Mice. Catalyst Journal. Vol. 4 No. 2: 72-78. http://doi.org/10.22216/jk.v4i2.4607
[5] Setioningsih, E., Setyaningsih, R and Susilowati, A 2004. Making Probiotic Drinks from Soy Milk with Lactobacillus casei, Lactobacillus plantarum, and Lactobacillus acidophilus inoculums. Biotechnology 1 (1): 1-6, May 2004, ISSN: 0216-6887, DOI: 10.13057/biotek/c010101
[6] Gibson, G.R and M.B. Roberfroid. 2008. Prebiotics: Concept, Definition, Criteria, Methodologies, and Products. Handbook of Prebiotics. CRC Press. New York.
[7] Harti A.S., Dwi Susi Haryati, Sunarto, Wiwik Setyaningsih, Sri Yatmihatun, 2015. The Potential Chito-Oligo-saccharide (COS) as Natural Prebiotic and Preservatives on Synbiotic Tofu in Indonesia. International Journal of Pharma Medicine and Biological Science ISBN 2278-5221. Vol. 4, No.3, July 2015, page 204 – 208.
[8] Hardiningsih, R and Nurhidayatm N, 2006. Effect of Hypercholesterolemia Feeding on Body Weight of Wistar White Rats Given Lactic Acid Bacteria. Biodiversity. Vol 7 no. 2: 127 – 130. DOI: 10.13057/biodiv/d070207
[9] Harti A.S., Anis Nurhidayati, Desy Handayani, Estuningsih, Heni Nur Kusumawati, Dwi Susi Haryati, 2014. The Fortification Tempeh of Rice bran Chitosan as Functional Food Antihypercholesterolemia in Indonesia. International Journal of Bioscience, Biochemistry and Bioinformatics. ISSN 2010-3638. Volume 4 No. 5, September 2014 pages : 423-427.
[10] Hermawan, Amrul Wahyu and Prima Retno Wikandari. 2016. Effect of Lactic Acid Bacteria Starter Culture Type on Soyghurt Characteristics. Surabaya: Department of Chemistry FMIPA Surabaya State University.
[11] Hidayati, Darimiyya. 2010. Lactic Acid Bacteria Growth Pattern during Soy Milk Fermentation. Journal of Agricultural Product Technology. Vol.3, No.2. pp 72-76.
[12] Huda, Mar'atul and Prima Retno Wikandari. 2016. Determination of β-Glucosidase Activity in Soybean Juice Fermentation with Lactobacillus plantarum B1765 Starter Culture. Surabaya: Department of Chemistry FMIPA Surabaya State University.
[13] Ministry of Health of the Republic of Indonesia 2018. Situation of Short Toddlers (Stunting) in Indonesia.
[14] Ministry of Health, R.I. 2018. Indonesian Basic Health Research 2018. Jakarta, Indonesian Ministry of Health
[15] Ministry of Health of the Republic of Indonesia. 2021. Pocket Book of Study Results on the Nutritional Status of Indonesia at the National, Provincial and District Levels.
[16] Koshi et al. 2022. Are Early Childhood Stunting and Catch-Up Growth Associated With School Age Cognition?-Evidence From an Indian Birth Cohort. Journal Plos One : March 2 2022.
[17] Kusumaningrum, E.N.. 2004. Making Soygurt Drink from Sari Tempe using Lactobacillus plantarum Journal of Mathematics, Science and Technology, Vol. 5 No. 1, March 2004, pp 64-75
[18] Lin, Shih-Bin; Chen, Shan-He; Peng, Kou-Cheng, 2009. Preparation of antibacterial chito-oligosaccharide by altering the degree of deacetylation of p-chitosan in a Trichoderma harzianum chitinase-hydrolysing process. Journal of the Science of Food and Agriculture, Vol. 89, No. 2, 30 January 2009, pp. 238-244

[19] Lin, Hua V., Andrea Frassetto, Edward J. Kowalik Jr, Andrea R. Nawrocki, Mofei M. Lu, Jennifer R. Kosinski, James A. Hubert, Daphne Szeto, Xiaorui Yao, Gail Forrest, Donald J. Marsh.2012 . Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3- Independent Mechanisms. SCFAs Regulate Gut Hormones and Obesity. Vol. 7, No. 4.

[20] Mahim, R., Akram, S., Leila, J., Naser, B., & Kavosi Ali. (2017). The Effect of Probiotic Yogurt on Blood Glucose and cardiovascular Biomarkers in Patients with Type II Diabetes : A Randomized Controlled Trial. Evidence Based Care Journal, (098 51), 25–35. https://doi.org/10.22038/ebcj.2016.7984

[21] Melliawati, R., C.D. Apridah, and Yopi. 2015. Selection of Lactic Acid Bacteria as Producers of Protease Enzymes. Pros. Sem. Nas. Mass. Biodiv. Indo. Vol. 1, No. 2. Pgs 184-188.

[22] Mulyaningsih, et al. 2021. Beyond Personal Factors: Multilevel determinants of Childhood Stunting in Indonesia. Journal Plos One : March 2 2022.

[23] Naidu, A. S., and Clemens, R. A. 2000. Probiotics In Natural Food Antimicrobial System. Boca Raton (US): CRC Press, LCC.

[24] Novák, P., and V. Havlícek. 2016. Proteomic Profiling and Analytical Chemistry: Protein Extraction and Precipitation. Prague: Institute of Microbiology, Academy of Science of the Czech Republic.

[25] Psichas, A., M.L. Sleeth, K.G. Murphy, L. Brooks, G. Bewick, A.C.Hanyaloglu, M.A. Ghatei, S.R. Bloom, & G. Frost. 2014. The Short Chain Fatty Acid Propionate Stimulates GLP-1 and PYY Secretion via Free Fatty Acid Receptor 2 in Rodents. International Journal of Obesity.

[26] Puspitasari, Kharisma Nur, and Prima Retno Wikandari. 2016. Potential of Lactobacillus plantarum B1765 as a Producer of SCFA in the Pikel Yakon (Smallanthus sonchifolius) Fermentation Process. Proceedings of the National Seminar on Chemistry and Learning. Surabaya: Department of Chemistry FMIPA Surabaya State University

[27] Putra, Noerman Y.P and Wikandari, P.R., 2020. The Effect Of Fermentation Time Against Total Bal And Ph Value In Making Of Synbiotics Soybean Extract. UNESA Journal of Chemistry, Vol 9 (1), 103 – 110.

[28] Pyo, Young-Hee, Tung-Ching Lee, and Young-Chul Lee. 2005. Enrichment of Bioactive Isoflavones in Soymilk Fermented with β-glucosidase-producing Lactic acid Bacteria. Food Research International. Vol. 38. Pgs 51-599.

[29] Soliman, et al. 2021. Early and Long-term Consequences of Nutritional Stunting From Childhood to Adulthood. Acta Biomed Vol 92 N 1 :2021168

[30] Sumarna. 2008. Changes of Raffinose and Stachyose in Soy Milk Fermentation by Lactic Acid Bacteria from Local Fermented Foods of Indonesia. Malaysian Journal of Microbiology. Vol. 4, No. 2. Pgs 26-34.

[31] Sumarna. 2009. Hydrolysis of Bioactive Isoflavone in Soymilk Fermented with β-Glucosidase Producing Lactic Acid Bacteria from Local Fermented Foods Of Indonesian. Malaysian Journal of Microbiology. Vol. 6, No. 1. Pgs 30-40.

[32] Widowati E, MAM Andriani, Amalia Putri K., 2011. Study of Total Probiotic Bacteria and Antioxidant Activity of Tempe Yoghurt with Substrate Variations. Journal of Agricultural Product Technology, Vol. IV, pp. 18-31; No. 1 February 2011. Faculty of Agriculture UNS Surakarta.

[33] Zahid, et al. 2020. Association between Child Snack and Beverage Consumption, Save Dental Caries and Malnutrition in Nepal. International Journal Of Environmental Research and Public Health.