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EFFECT OF CONSUMING PREBIOTIC BISCUIT BASED ON PROTEIN QUALITY EVALUATION BY IN VIVO METHOD

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ABSTRACT

Background: *Prebiotic biscuits are biscuits made from composite flour consisting of plaintain hump flour, yellow sweet potato flour, black soybean flour and black soybean sprouts flour. The combination of the resulting composite flour is known to have a higher nutritional value when compared to wheat flour. However, prebiotic biscuits contain certain ingredients such as protein or food fiber whose structure must be known in advance to modify their functional properties after consumption.*

Objective: *The evaluation of protein quality was carried out to determine the effect of giving "BonnisA" prebiotic biscuits with different protein sources in increasing the quality of protein in Vivo.*

Methods: *Research method used was experimental which was analyzed descriptively with 4 treatments 5 times. The object used in this experiment are male wistar rats (Sprague Dawley) weighing 150 - 220 g, and the treatments were given were black soybean flour prebiotic biscuits, prebiotic biscuits with black soybean sprouts flour, standard rations and egg white flour.*

Results: *The results obtained showed that the highest increase in body weight of rats was treated with the prebiotic biscuit BonnisA black soybean flour of 13.29%. Furthermore, the evaluation of protein quality on rats was increased in black soybean flour prebiotic biscuits based on true digestibility (TD) (99.21%), biological values (BV) (99.25%), and net protein utilization (NPU) (98.47%).*

Conclusions: *The evaluation of protein quality showed that there was a significant difference ($p < 0.05$) between the provision of black soybean flour prebiotic biscuits and prebiotic biscuits with black soybean sprouts flour and standard rations and egg white flour at true digestibility (TD), biological values (BV) and Net Protein Utilization (NPU).*

Keywords *: Prebiotic biscuits, Evaluation of protein quality, In Vivo*

INTRODUCTION

Biscuits are a snack containing 35% energy sufficiency needs, 0.8% protein, 1.1% fat and 5.4% carbohydrates in 25 g of biscuits.[1,2] However, excessive consumption of biscuits can make it difficult to meet balanced and adequate nutritional needs which causes some people to experience problems in nutritional balance that lead to diseases such as obesity, cholesterol, diabetes, hypertension. [3] Therefore, we need improvement to increase the nutritional value on biscuit product by using another ingredient with healthier compound by adding composite flour. So hopefully that improvement can reduce comsumption worriness while eating biscuit as a snack.

Composite flour are one of the flour from several commodity such a tubers, nuts, or cereals with the addition with or without wheat flour.[4] The combination of plaintain hump flour, yellow sweet potato flour and black soybean sprouts flour are one of composite flour. The combination of the three flours because each flour has good nutritional value, especially in prebiotics.[5] Prebiotics are considered as indigestible food ingredients that can have an effect on the body by stimulating growth or increasing the activity of bacteria in the large intestine.[6]

Prebiotic biscuits are biscuits made from composite flour consisting of plaintain hump flour, yellow sweet potato flour, black soybean flour and black soybean sprouts flour. Decision of using the combination of composite flour because it has a higher nutritional value than wheat flour. This can be indicated by an increase carbohydrate contain prebiotics (Fructooligosaccharide (FOS) and Inulin) by 85.68% from the addition of yellow sweet potato flour, 39.09% protein with the addition of black soybean sprouts flour [7] and fiber content of 29.62% due to the addition of plaintain hump flour.[8]

There are certain ingredients in functional food products. Such as prebiotics, antioxidants or dietary fiber, whose structure must be known in advance to modify the functional properties after consumption.[9] For example in the evaluation of protein quality. Evaluation of protein quality needs to be done on prebiotic biscuits because protein is very influential in the body because of its very important role for the body. Prebiotics have adhesion properties to the *mucosa* intestinal which acts as a barrier to pathogens thereby increasing the absorption of nutrients such as protein. Protein is a compound in food that is very important for the body because this compound functions as a source of energy and as a building and regulating substance.[6]

Based on the data above, prebiotic biscuits are known to have good effects on health because they contain more than one nutritional components. And it claimed can improve the protein absorption on the body, so the value of protein quality in prebiotic biscuits needs to be known in vivo.

METHOD

The research was conducted in October 2020 at 2 different laboratory, such as Therapy and Pharmacology Laboratory for rats control and Food Technology Laboratory for protein quality research with Kjehdahl method at Padjadjaran University (UNPAD). This research has approved by Research Ethics Committee (No Register. 0718071040). The object experiment used were 20 male wistar rats (*Sprague Dawley*) weighing 150 - 220 g with the treatment that will be carried out on the sample is:

A = Egg White Flour
B = Standard ration
C = Black Soybean Flour Prebiotic Biscuits (contain 70 g of composite flour, the blends containing Black Soybean Flour)
D = Black Soybean Sprouts Flour Prebiotic Biscuits (contain 70 g of composite flour, the blends containing Black Soybean Sprouts Flour)

The research method used was an experimental method which was data analyzed descriptively, followed by a statistical paired sample T-test to determine the effect of treatment variations on the body weight of the rats, and for the value of protein quality in prebiotics using One Way ANOVA. The data will be inputted and processed using *Statistical Product Service Solution* software and the result were displayed in statistic data on the table.

Making Prebiotic Biscuits[9]

A total of 24 g of water, 35 g of refined sugar, 0.4 g of salt, margarine, 0.35 g of baking soda, and 0.25 g of baking powder are mixed using a mixer for 10 minutes at high speed. Then add 20 g of full cream milk and 15 g of egg yolk and knead with a composite flour consisting of 27.5 g of plaintain hump flour, 22.5 g of yellow sweet potato flour, and 20 g of black soybean flour until smooth. Print the dough and age it for 10 minutes at room temperature of 25^{o} C, then bake it in the oven at 150^{o} C for 8 minutes.

Weighing Rat Weight [10]

A total of 20 wistar rats (*Sprague dawley*) were put in cages and given an adaptation period of 7 days. Rats were fed a standard ration and drinking water, given ad libitum feed and drinking water in a separate place. After going through the adaptation period the rat were divided into 4 groups based on treatment, then a 28-day trial period was applied and the treatment was given by means of sonde. Weighing the rats body weight using a scale to determine the difference after being given a treatment ration in the form of increase or decrease in body weight of the rats.

The Kjehdahl method [11]

The kjehdahl method was used to determine nitrogen levels in feces and urine, and to determine the levels of protein contained in samples of egg white flour, black soybean flour biscuits and black soybean flour biscuits. Weigh the feces sample that has been mashed as much as 1 gram and then put it in the kjehdahl flask. Add the kjehdahl tablet to the Kjeldahl flask containing stool and urine samples and followed by adding 10 ml of H2SO4 solution, the addition of H2SO4 is carried out in a fume hood. Carry out the digestion process in the acid chamber by heating the sample using an electric stove on the Kjeldahl flask until it turns clear, then cooling the Kjeldahl flask for 20 minutes. Add 25 ml of distilled water and 40% NaOH reagent to the Kjeldahl flask. Add 30 ml of H3BO3 to the sample and 2 drops of methyl red indicator to hold the distillate from the distillation. Do the distillation using a distillator, add NaOH reagent to the sample and do the distillation until the sample changes color to Tosca. Titrate with the HCL solution until the color turns pink.

In Vivo Protein Quality Testing [10]

The pre-treatment was carried out by giving a standard ration of 30 g for 7 days. The experimental period was given for 28 days with each treatment given to 4 groups of rats. The sample is given as much as 5 grams in 100 ml / day. Stool and urine collection separately for each of 2 rats from 4 groups of rats in the last 7 days of treatment. This action in this experiment because this research used biological instrumental as an object experiment, and to avoid non-uniformity and

prejudice in research results.

Testing nitrogen levels in the feces and urine of rats using the Kjehdahl macro method. The feces are dried using an oven while the urine is not given any treatment and H2SO4 is added. Values were obtained based on the weight of dried rat faeces and urine volume in rats during the last 7 days.

Level of N (%)
100% x ((Total HCl sample – Total HCl blanco) x HCl x 0.014)/(Total Sample)

Protein content (%)
Total nitrogen (%) x conversion factor
Note: conversion factor = 6.25

True Digestibility (TD)
100 - (N consumption - (N faecal -N metabolic)) / (N digestibility) x 100

Biological Value (BV)
100 - (N consumption - (N faecal - N metabolik) - (N urine -N endogen)) / (N digestibility - (N faecal -N metabolic))

Net Protein Utilization (NPU)
NPU (%) = TD x BV

RESULTS

Rats Weight Increases

Based on the results of the T test shown in Table 1, the treatment of black soybean flour prebiotic biscuits (C) on the weight gain of rats had no significant effect when compared to the comparison treatment (A and B). In Table 1, there is data on weight gain in different rats, rats treated with egg white flour (A) have an insignificant increase in body weight with a percentage (of) 3.72% of body weight, this also occurs in rats with treatment of BonnisA biscuits black soybean flour (C) was 13.29% body weight and BonnisA biscuits black soybean flour (D) 1.12% body weight. However, there was a significant increase in body weight of the rats treated with the standard ration (B) of 9.23%.

Rat weight increases can be one of indication that influence the utilization of protein for growth. Rat weight increases can conclude there is an availability of protein in rat body. Results can be affected by given a different treatment, and the result display in grams of weight body

Table 1.Rat Weight Increases After Treatment

Weight Rats (g)	Treatment			
	A	**B**	**C**	**D**
Before	209.6000 ± 9.55a	197.2000 ± 8.32[a]	186.6000 ± 4.320[a]	178.4000 ± 7.215[a]
After	217.4000 ± 20.13	215.4000 ± 12.74[a]	211.4000 ± 31.20[a]	176.000 ± 8.400[a]
p-value	0.761	0.213	0.094	0.884

Rats weight increases can be an indication in determining the effect of feeding samples on rats. The increases in body weight of rats can be a factor that influences the utilization of protein for growth.
Note: The average treatment which is marked with the same row shows the results that are not significantly different based on the paired sample T test.

Nitrogen Levels in Feces and Urine

Table 2. Nitrogen levels Contain in Feces and Urine

Treatment	Nitrogen levels	
	Feces	**Urine**
A2	2.83 ± 0.52	0.23 ± 0.17
A3	1.66 ± 0.44	0.12 ± 0.15
B6	0.4 ± 0.07	0.11 ± 0.15
B7	0.41 ± 0.16	0.05 ± 0.04
C2	0.53 ± 0.04	0.09 ± 0.13
C5	0.5 ± 0.03	0.08 ± 0.06
D2	0.48 ± 0.06	0.04 ± 0.04
D3	0.35 ± 0.13	0.02 ± 0.01
p-value	0.000	

Nitrogen contained and excreted through urine is keratin, urea, ammonia, allantoin and amino acids. Testing of nitrogen levels in feces and urine in rats was carried out using the macro Kjehdahl method. The results of testing fecal and urine nitrogen levels in percent.

Based on the data in Table 2, nitrogen levels in feces and urine have different numbers. The highest feces and urine nitrogen level was found in A2 rats (2.83% and 0.23), A3 rats (1.66% and 0.12%) with the treatment of egg white flour followed by the fecal nitrogen content of the rats treated with black soybean flour prebiotic biscuits C2 rats (0.53% and 0.09%), C5 rats (0.5% and

0.08%) then the treatment feces and urine nitrogen level black soybean sprouts flour prebiotic biscuits D2 rats (0.48% and 0.04%) and D3 rats (0.35% and 0.02%). While the lowest levels of feces and urine nitrogen level were found on the treatment of standard rations B6 rats (0.4% and 0.11%) then B7 ratss (0.41% and 0.05%).

Protein Levels and Nitrogen Levels of Treatment Samples

Based on the data in Table 3, egg white flour has a protein content of 81.82% wb and 86.11% db, and a nitrogen content of 13.78%. The standard diet has a protein content of 7.43% wb and 8.64% db with a nitrogen content of 1.38%. The protein content contained in the prebiotic biscuits of black soybean flour was 8.21% wb and 8.46% db and nitrogen content was 1.35%. The prebiotic biscuits of black soybean sprouts flour had a protein content of 9.40% wb and 9.72% db with a nitrogen content of 1.56%.

Table 3. Protein Content and Nitrogen Content of Treatment Samples

No.	Treatment Samples	Protein Content (%)		N (%)
		wb (%)	db (%)	
1	**Egg White Flour (A)**	81.82 ± 2.78	86.11 ± 2.92	13.78 ± 0.47
2	**Standard Ration (B)**	7.43 ± 1.48	8.64 ± 1.73	1.38 ± 0.28
3	**Black soybean flour prebiotic biscuits (C)**	8.21 ± 0.09	8.46 ± 0.09	1.35 ± 0.01
4	**Black soybean sprouts flour prebiotic biscuits (D)**	9.40 ± 3.68	9.72 ± 3.81	1.56 ± 0.61
	p Value		0.003	

The protein content of egg white flour, standard rations, black soybean flour prebiotic biscuit and black soybean sprouts flour prebiotic biscuit were carried out using the kjehdahl method to obtain nitrogen content in food samples.

Evaluation of Protein Quality Value

True Digestibility

Based on the One Way Anova test, it can be concluded that some true digestibility in each treatment is significantly different (p-value <0.05) and based on the Duncan test on the Table 4, the digestibility of treatment B, C, D is significantly different from treatment A. Treated rats were given prebiotic biscuits with black soybean flour (C5) have a highest true digestibility which is 99.21%. The lowest digestibility values were rats treated with egg white flour (A3) at 87.94%.

Table 4. True Digestibility In Protein Quality

Sample	TD
A2	89.23 ± 0.57a
A3	87.94 ± 0.48b
B6	98.98 ± 0.15cd
B7	98.89 ± 0.32cd
C2	98.85 ± 0.07cd
C5	99.21 ± 0.07c
D2	98.93 ± 0.13cd
D3	98.67 ± 0.23d
p-value	0,007

The value of true digestibility (true protein digestibility) is an indicator of the amount of nitrogen or protein that the body absorbs from food. The true digestibility value is also considered as the percentage of protein contained in food materials which is converted into absorbable protein in the body.

Note: The average treatment which is marked with the same lowercase letter shows results that are not significantly different based on the One Way Anova test (p-value).

Biological Value

Based on the One Way Anova test, it can be concluded that some of biological value of each treatment is significantly different (p-value < 0.05) and based on the Duncan test on the Table 5, the biological value of treatment B, C, D is significantly different from treatment A. Based on the data in Table 5 the highest biological value (BV) was found in rats treated with prebiotic biscuits with black soybean flour (C5) which is 99.25%, and the lowest values were rats treated with egg white flour (A3) 89.26%.

Table 5. Biological Value In Protein Quality

Sample	BV
A2	93.00 ± 1.54a
A3	89.26 ± 1.33b
B6	99.01 ± 0.36c
B7	98.72 ± 0.49c
C2	98.64 ± 0.21c
C5	99.25 ± 0.06c
D2	98.90 ± 0.22c
D3	98.43 ± 0.39c
p-value	0.000

Biological value determined by percentage value of absorbed protein that is converted into body protein. The higher the biological value of a food, the higher the protein that is absorbed and converted into body protein. Result display by using calculation and protein content on rats feces and urine.

Note: The average treatment which is marked with the same lowercase letter shows results that are not significantly different based on the One Way Anova test (p-value).

Net Protein Utilization

Based on the results of the One Way Anova test, some of the NPU value for each treatment was significantly different (p-value <0.05). Based on the Duncan test on the Table 6, NPU for treatment B, C, D was significantly different from treatment A. The highest NPU was found in C5 rats treated with prebiotic biscuits of black soybean flour, and the lowest NPU values were in rats treated with egg white flour (A3) 78.5%.

Table 6. Net Protein Utilization In Protein Quality

Sample	NPU
A2	82.99 ± 1.90a
A3	78.50 ± 1.60b
B6	98.00 ± 0.50c
B7	97.63 ± 0.80c
C2	97.50 ± 0.25c
C5	98.47 ± 0.11c
D2	97.85 ± 0.35c
D3	97.12 ± 0.60c
p-value	0.000

Net protein utilization (NPU) determined by measure protein quality using a comparison of the digestibility values between proteins, namely paying attention to the amount of protein retained and the amount of protein that can be digested**.** NPU value can decided by using calculation.

Note: The average treatment which is marked with the same lowercase letter shows results that are not significantly different based on the One Way Anova test (p-value).

DISCUSSION

Rats Weight Increases

Weight gain in rats can be an indication in determining the effect of feeding samples on rats. The samples given to rats were prebiotic biscuits of black soybean flour (C), prebiotic biscuits of black soybean flour (D) and egg white flour (A), standard ration (B) as a comparison. This weight gain occurs because it is influenced by the amount of feed consumed by the rats.[12]

The increase in body weight of rats is a factor that affects the utilization of protein for growth. In rats treated with prebiotic biscuits, black soybean sprouts flour (D) experienced a decrease in body weight, this is because the germination process in black soybean sprouts increased the levels of isoflavones in prebiotic biscuits.[13]

Isoflavones in soybeans are known to be non-nutritive compounds. The levels of isoflavones contained in soybeans, which contain flavonoids, can inhibit the weight gain of rats. So it can be ascertained that the changes that occur are the weight loss of rats, because in addition to the flavonoid content it can also be caused by the unsaturated fat content in soy which can reduce body weight.[14]

Quantitative measurements of protein metabolism are generated from the calculation of nitrogen in food and excretion products (feces and urine) and to determine the increase or decrease in nitrogen levels in the animal's body. [15]

Based on the data in Table 2, nitrogen levels in feces and urine have different numbers. Nitrogen levels vary due to nitrogen excreted through feces depending on the results of digestion by microbes and the efficiency of maintaining

bacteria.[15] There are many factors that can affect nitrogen excretion through feces, namely animal body weight, dry matter consumption, crude fiber content, energy and protein rations as well as digestive metabolism, the type of food consumed and the digestive tract. [16]

The high fecal nitrogen in A2 and A3 rats was due to the high content of crude fiber and low digestibility in these treatments (Table 2) compared to treatments B, C and D. because of the high digestibility in B6 rats and B7 rats (Table 2). The excretion of nitrogen from feces is influenced by several factors such as the level of protein contained in food ingredients, the value of digestibility and energy levels.[17] Nitrogen contained in feces is also influenced by feed protein that is not digested by rats, N-endogenous consists of digestive enzymes and other fluids that are excreted into the digestive tract, besides that eroded mucosal cells contain protein and microbes in the digestive tract in the digestive tract. [18]

The amount of nitrogen produced by rat urine in treatment A2 and A3 was caused by high levels of protein in treatment A, namely egg white flour (Table 6). In addition, the degradation of protein in the rumen which forms ammonia is utilized by microbes to form microbial protein and the rest passes into the blood vessels, into the liver and then excreted through urine quickly.[18] Nitrogen contained in urine is influenced by several factors, namely nitrogen consumption, nitrogen absorption in rats, ration protein levels, protein digestibility and physical form and kinds of food ingredients.[19]

Nitrogen is an element that is contained in protein and is not available in a group of other major chemical compounds that the body needs (carbohydrates and fats).[20] Nitrogen contained in urine is influenced by several factors, namely nitrogen consumption, nitrogen absorption in rats, ration protein levels, protein digestibility and physical form and kinds of food ingredients.[19]

Protein Levels and Nitrogen Levels of Treatment Samples

Nitrogen content in food sources greatly affects the level of nitrogen consumption and digestibility in rats. Based on the data in Table 3, the protein content and nitrogen content of the treatment samples have various numbers. According to SNI 01-4323-1996 the requirements for protein content in egg flour are at least 75% bw [21], then the test results for the protein content of egg white flour are appropriate. This is because 60% of the components of an egg are egg white, egg white contains 5 types of protein and a few carbohydrates. It is also known that when egg white is processed into flour it can increase the protein content in the product itself.[22]

The amount of protein content in the standard ration is known because of the many composition of the ration. The composition of the standard rations consists of: corn, dadak, fish meal, soybean meal, coconut meal, meat meal, bone meal, wheat fractions, peanut meal, leaf flour, canola, vitamins, calcium, pashate, trace minerals and antioxidants.[23] According to AOAC in 1990, the standard ration contains 10% protein, 8% oil, 5% salt and mineral mixture, 1% vitamin mixture, 1% cellulose, and 5% water.[23]

The protein content in the two prebiotic biscuits has met SNI 01-2973-2011 that biscuits have a protein content of at least 5%.[9] The protein content is high because most of the composition of the prebiotic biscuits consists of a mixture of plaintain hump flour, yellow sweet potato flour, and black soybean flour. Plaintain hump flour is known to have a protein content of 3.40% [24], yellow sweet potato flour contains 2.11% protein [25], black soybean flour has a protein content of 46.10%. [26]

The prebiotic biscuits of black soybean sprouts flour have a different composition, namely the black soybean sprouts flour. Black soybean sprouts flour has a higher protein content compared to black soybean flour, which is 54.67% [26] This is because the germination process has been shown to increase the yield in flour, this research has been carried out on the manufacture of comak bean flour, whose initial protein content is 14.01% after being germinated to 27.77%. Therefore, the protein content in the prebiotic biscuits of black soybean sprouts flour is higher than the prebiotic biscuits of black soybean flour.

Evaluation of Protein Quality Value

In Vivo evaluation of protein quality values by measuring nitrogen balance (NPU) is known to be a more accurate method compared to the analysis method for amino acid levels to determine nutritional value. The total nitrogen utilization value (NPU) includes true digestibility (TD) and biological value (BV). The following is the data from the evaluation of protein quality values by In Vivo method.

True digestibility and biological value in the rats treated with black soybean flour prebiotic biscuits (C5) more higher than other treatments because the high levels of protein on it's ration which is black soybean flour prebiotic biscuits that impacted to protein absorbtion on rats body. Based on Table 3, black soybean flour prebiotic biscuits have a 8.21% wb and 8.46% db protein content and nitrogen level was 1.35%. This can be supported by statement total nitrogen consumption, nitrogen

absorption in the rat's body, the level protein on the ration, and protein digestibility and the type of food ingredients can impacted the value of true digestibility.[27] Also it can be the effect of the ingredient in prebiotic biscuit, such a composite flour. Plaintain hump flour known containing oligosaccharides (fructooligosaccharides (FOS)) and Inulin.[3] There is known have a fiber content of 52.92% which consists of oligosaccharides such as inulin, FOS and GOS or we can said prebiotic.[28] It is known that a food ingredient containing prebiotics have good protein quality if the true protein digestibility value is more than 90%.[29] Therefore, food can considered capable of providing good growth if it is consumed in sufficient quantities and energy consumption is sufficient if it has a biological value of 70% or more. [30]

Net protein utilization (NPU) is one way to measure protein quality using a comparison of the digestibility values between proteins, namely paying attention to the amount of protein retained and the amount of protein that can be digested.[31] The NPU value is closely related to the value of digestibility and biological value, because the NPU value includes all nitrogen that can be used by the body.[32] This can be proven in Table 6 that digestibility, biological value, and NPU have the same results. From the attached NPU value, it can be interpreted that the prebiotic biscuits of black soybean flour (C) and prebiotic biscuits of black soybean sprouts flour (D) have better nutritional value than the standard ration samples and egg white flour.[31]The higher the NPU value in a food, the more nitrogen in that food can be retained in the body.The results obtained are in accordance with research conducted by Hussain et al. 2012 which states that the treatment of bread with substitution of wheat flour with 16% hemp flour has a greater NPU value of 54.65% compared to bread with 100% wheat flour of 44.30. %.[33]

From this research we can concluded the advantages and disadvantages from using this method (In vivo). The advantage of this research is that it uses living creatures (rats) as experimental objects. With this method, we can get a real results from the amount of protein that can be digested by consuming prebiotics when compared using the in vitro method, it because the results based on actual from data process digestive system in rats body. Meanwhile, the disadvantages from using in vivo method is that we cannot get stable results when compared with results data using in vitro methods, it because we cannot predict the biological factors that exist in living research objects.

CONCLUSION

Black soybean flour prebiotic biscuits can be used to be source of protein consume from prebiotic content inside the biscuit based on evaluation of protein quality value results, such as total nitrogen utilization value (NPU) includes true digestibility (TD) and biological value (BV).

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REFERENCE

1. Hamidah N, Legowo AM, Anwar S. Tepung ubi kayu (manihot esculenta) dan tepung tempe kedelai mempengaruhi pengembangan volume dan mutu gizi protein roti tawar. Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition). 8 Desember 2016;4(1):55–62.
2. Linda D, Aristi A, Rasni H, Susumaningrum LA, Susanto T, Siswoyo S. The Relationship Between High Sodium Food Consumption and The Incidence of Hypertension Among Farm Workers at Public Health Centre of Panti in Jember Regency. Buletin Penelitian Sistem Kesehatan. 2020;
3. Wardani AK, Wardani IR. Eksplorasi Potensi Kedelai Hitam Untuk Produksi Minuman Fungsional Sebagai Upaya Meningkatkan Kesehatan Masyarakat. Jurnal Pangan dan Agroindustri. 2014;2(4):58–67.
4. Pudjihastuti I, Supriyo E, Devara HR. PENGARUH RASIO BAHAN BAKU TEPUNG KOMPOSIT (UBI KAYU, JAGUNG DAN KEDELAI HITAM) PADA KUALITAS PEMBUATAN BERAS ANALOG. Gema Teknologi. 30 April 2021;21(2):61–6.
5. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, dkk. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology. 2017.
6. Mamat H, Hill S. Structural and functional properties of major ingredients of biscuit. International food research journal. 2018;25(2).
7. Saragih B, Mulawarman U. Analisis Mutu Tepung Bonggol Pisang Dari Berbagai Varietas Dan. Jurnal TIBBS Teknologi Industri Boga dan Busana. 2014;9(1):22-29(March 2013).

8. Csapó J, Albert C, Szigeti TJ. Functional foods. Elelmiszervizsgalati Kozlemenyek. 2019;65(1).
9. Sumanti DM, Setiasih IS, Balia RL, Karuniawan A, Lengkey HAW. BIOACTIVE COMPOUNDS AND FUNCTIONAL PROPERTIES OF SOME FLOUR COMPOSITES IN THE MANUFACTURING OF FUNCTIONAL FOOD PRODUCTS. 2017;17(3).
10. Astawan M, Mursyid M, Muchtadi D, Wresdiyati T, Bintari SH, Suwarno M. Evaluasi Nilai Gizi Protein Tepung Tempe yang Terbuat dari Varietas Kedelai Impor dan Lokal Evaluation on Protein Nutritional Value of Tempe Flour Made from Imported and Local Soybean Varieties. JURNAL PANGAN. 1 Maret 2014;23(1):33–42.
11. Feldsine P, Abeyta C, Andrews WH. AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis. Journal of AOAC INTERNATIONAL. 1 September 2002;85(5):1187–200.
12. Taylor MJ, Tanna S, Sahota T. In vivo study of a polymeric glucose-sensitive insulin delivery system using a rat model. Journal of pharmaceutical sciences. 2010;99(10):4215–27.
13. Abutheraa R, Hettiarachchy N, Kumar-Phillips G, Horax R, Chen P, Morawicki R, dkk. Antimicrobial activities of phenolic extracts derived from seed coats of selected soybean varieties. Journal of Food Science. 2017;82(3):731–7.
14. Rice-Evans C. Flavonoid antioxidants. Current medicinal chemistry. 2001;8(7):797–807.
15. Van Soest PJ. Nutritional ecology of the ruminant. Cornell university press; 2018.
16. Ayman A. Cambridge IGCSE Biology Coursebook (third edition) public 20% (2). [dikutip 2 Agustus 2023]; Tersedia pada: https://www.academia.edu/89869388/Cambridge_IGCSE_Biology_Coursebook_third_edition_public_20_2_
17. Koenig J, Boling J, Bull L. Energy and protein metabolism in ewes as influenced by age and dietary protein-calorie ratio. Journal of Animal Science. 1980;51(4):1011–22.
18. Delgado Andrade C, Roncero Ramos I, Alonso-Olalla R, Seiquer I, Navarro MP. Maillard product consumption and nitrogen digestibility in young and adult rats. 2014;
19. Clark JE, Hellgren EC, Parsons JL, Jorgensen EE, Engle DM, Leslie DM. Nitrogen outputs from fecal and urine deposition of small mammals: Implications for nitrogen cycling. Oecologia. 2005;
20. Gomez IJ, Arnaiz B, Cacioppo M, Arcudi F, Prato M. Nitrogen-doped carbon nanodots for bioimaging and delivery of paclitaxel. Journal of Materials Chemistry B. 2018;6(35):5540–8.
21. Anukiruthika T, Moses J, Anandharamakrishnan C. 3D printing of egg yolk and white with rice flour blends. Journal of Food Engineering. 2020;265:109691.
22. Bun K, Informatika JT, Industri FT, Gunadarma U, Barat J. TELUR. Jurnal Ilmiah Teknologi dan Rekayasa. 2018;
23. International A. AOAC: Official Methods of Analytical Chemist. Assoc of Official Anal Chem. 1990;
24. Kaushal M, Swennen R, Mahuku G. Unlocking the microbiome communities of banana (Musa spp.) under disease stressed (fusarium wilt) and non-stressed conditions. Microorganisms. 2020;
25. Ginting E, Antarlina SS, Widowati S. Soybean Superior Varieties for Food Industry Raw Materials (Original title in Indonesian: Varietas Unggul Kedelai untuk Bahan Baku Industri Pangan). Jurnal Litbang Pertanian. 2009;
26. Astawan, M & Hazmi K. Karakteristik fisikokimia tepung kecambah kedelai dan tepung kedelai. Artikel Departemen Ilmu dan Teknologi Pangan, Fakultas Teknologi Pertanian, IPB. 2016;
27. Moorhead LC, Souza L, Habeck CW, Lindroth RL, Classen AT. Small mammal activity alters plant community composition and microbial activity in an old-field ecosystem. Ecosphere. Mei 2017;8(5):e01777.
28. Kase EP. PENGARUH SUBSTITUSI JAGUNG GILING OLEH TEPUNG BONGGOL PISANG FERMENTASI DALAM PAKAN KONSENTRATTERHADAP KANDUNGAN DAN KECERNAAN NUTRIEN SECARA IN VITRO. 2020;
29. Bröer S. Amino acid transport across mammalian intestinal and renal epithelia. Physiological Reviews. 2008.
30. Almatsier S. Prinsip Dasar Ilmu Gizi. Jakarta: Gramedia Pustaka Utama; 2001.
31. Hughes GJ, Ryan DJ, Mukherjea R, Schasteen CS. Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation. Journal of Agricultural and Food Chemistry. 2011;
32. Tome D, Bos C. Dietary protein and nitrogen utilization. Dalam: Journal of Nutrition. 2000.

33. Hussain S, Anjum FM, Butt MS, Alamri MS, Khan MR. Biochemical and nutritional evaluation of unleavened flat: Breads fortified with healthy flaxseed. International Journal of Agriculture and Biology. 2012;14(2):190–6.