ISSN: 2347-467X, Vol. 07, No. (3) 2019, Pg. 828-841

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

The Effect of *Tempeh Gembus* Substitution on Protein Content, Calcium, Protein Digestibility and Organoleptic Quality of Meatballs

AUDREY MUTIARA SETYA ARINI[1], DIANA NUR AFIFAH[1]* and FILLAH FITHRA DIENY[1]

Department of Nutrition Science, Diponegoro University, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.

Abstract

Osteopenia is a sign of osteoporosis that usually occurs in adolescent aged 18-24 years. One of the efforts to manage osteopenia is by giving a high calcium diet. Meatballs substituted by *tempeh gembus* can be used as an alternative to local food-based snacks as source of calcium. To analyze the effect of the substitution of *tempeh gembus* on the protein content, calcium, digestibility of the protein, and the acceptability of meatballs. The study was conducted in two stages, there are preliminary study and main study, and using randomized single factor experimental, *tempeh gembus* levels (25%, 50%, 75%, 100%) and control (0% *tempeh gembus*). The analysis of the content of protein was conducted using *Kjeldahl* method, calcium contents using Uv-Vis spectrophotometer, and protein digestibility using *in-vitro* method. Data was analyzed with *mann-whitney.* Organoleptic tests were carried out by hedonic testing of 30 semi-trained panelists. Substitution of *tempeh gembus* had significant effect on the protein contents and protein digestibility, but not significant effect on the calcium contents. Formulation with 25% *tempeh gembus* substitution was found to be a best formulation of meatballs containing protein content of 8.03%, calcium contents of 351.19 mg / 100 g, and protein digestibility of 53.22%. Meatball with 25% *tempeh gembus* with 78 mg per serving is sufficient for nutrition label reference in general category, 13% energy; protein by 11%; calcium by 25%.

Article History

Received: 27 July 2019
Accepted: 19 November 2019

Keywords

Meatballs;
Nutrient Content;
Substitution;
Tempeh Gembus.

CONTACT Diana Nur Afifah dianaurafifah@live.undip.ac.id Department of Nutrition Science, Diponegoro University, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.

© 2019 The Author(s). Published by Enviro Research Publishers.
This is an Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).
Doi: http://dx.doi.org/10.12944/CRNFSJ.7.3.22

Introduction

Osteoporosis is a bone metabolic disease characterized by a decrease in bone mass due to reduced bone matrix and minerals accompanied by microarchitecture damage from bone tissue, with a consequent decrease in bone strength, resulting in a tendency for bones to break easily.[1] Osteoporosis is a disease that is classified as a silent disease because it does not show specific symptoms.[2].Osteopenia is a sign of osteoporosis that begins with low bone density, if it lasts for a long time it can lead to decreased bone strength resulting in osteoporosis.[3]

A lifestyle that occurs in adolescent aged 18-24 years such as smoking, consumption of different types of baverages, not exercising, not drinking milk, sedentary lifestyle, consumption of fast food which is generally high in protein and inappropiate calcium intake are the factors lead to an increase the risk of osteoporosis at this time.[4] Research in high school adolescents showed that 76.2% of adolescents are in a situation of lack of calcium consuming category. The average calcium intake is only 559.05 mg/day or 55.9% of the Daily Value (DV).[5]

Calcium intake recommended for women who have not yet menopause is around 1000-1200 mg /day.[6] The main sources of calcium found in foods are milk and other dairy products such as cheese or yogurt, green vegetables such as broccoli, fortified juices with calcium, and soybeans and soy products.[7] Indonesia has a variety of soy products such as soybean milk, tofu, tempeh, soy sauce, tauco, and *tempeh gembus* which can be an alternative affordable source of calcium.[8]

Meatballs are a type of food that is shaped like a ball made from meat and flour. In general, meatballs in the community vary in name according to the types of basic ingredients such as fish meatballs, chicken meatballs, and beef meatballs or original meatball.[9]

Tempeh gembus is one of the functional food products that is widely eaten by Indonesians. *Tempeh gembus* are usually made from tofu residue from soybeans which are commonly used as animal feed. When compared with other soybean products, such as tofu and tempeh, *tempeh gembus* has relatively a little nutrient content. This is because *tempeh gembus* is the residual of tofu processing. Protein loss in tofu processing is mainly due to boiling process and unused residue from tofu.[10]

In 2002, Murdiati reported that tofu residue contains 22.28% protein, 5.87% fat, and 71.83% carbohydrates.[11] Another study showed that an innovative snack product like kerupuk made from tapioca flour combined with *tempeh gembus* still contains high fiber ranged between 38.1 to 67%.[12] *Tempeh gembus* contains of essential fatty acids, such as linoleic acid 21.51%, linolenic acid 1.82% and unsaturated fatty acids 16.72%.[13] *Tempeh gembus* (dry weight per 100 g) also had some important nutrition contents, such as protein 4.07 g, carbohydrate 14.25 g, fiber 4.69 g, calcium 159.98 mg and iron 0.48 mg.[14] *Tempeh gembus* had health effects such as proteolytic, fibrinolytic and anti-imflammation effect that might be able to act as antithrombotic.[15-19] Based on the previous study, *tempeh gembus* also known had antimicrobial[13] (against *S. aureus*, *B. subtilitis,* and *S. mutans*) and antioxidant activities.[20] Raw *tempeh gembus* contains fiber 3.93 g, calcium 143 mg, phosphorus 50 mg, and iron 0.40 mg per 100 g.[21] The content of these nutrients will also be easier to digest due to fermentation. The digestive enzymes produced by tempeh mold make protein, fat, and carbohydrates in tempeh easier to digest.[22] Protein digestibility values of tofu residue are also quite high even though it is still lower than tofu. Protein digestibility on soybean yield was 78.7% for tofu residue, 92.7% for tofu and 65.3% for boiled soybeans.[11]

Analysis of nutrient content in the form of calcium and protein contents was carried out to determine the nutritional content of *tempeh gembus* meatballs. Protein digestibility test was carried out to determine the quality of protein digestibility in *tempeh gembus* meatballs. The acceptance level test was conducted to test the acceptance of *tempeh gembus* meatball products among teenagers. Hence, the objective of the present work was found the best formulation of *tempeh gembus* meatballs and analyze the effect of the substitution of *tempeh gembus* on the protein content, calcium, digestibility of the protein, and the acceptability of *tempeh gembus* meatballs.

Materials and Methods

Materials of this study sample used *tempeh gembus* which is obtained from the manufacturer located in Semarang Indah Cluster. *Tempeh gembus* used was from the local soybean processing in the Grobogan area.

This research was an experimental study with a complete randomized one-factor design. The factor in this research is the comparison of the composition of *tempeh gembus* with beef in units of percent. In this study, 4 levels of treatment were carried out with a percentage of 25%, 50%, 75%, 100% and 1 control with a percentage of 0%. Each group was repeated five times. All samples will be analyzed for protein contents was conducted using *Kjeldahl* method, calcium contents using spectrophotometer Uv-Vis, protein digestibility using *in-vitro* method, and organoleptic tests on one sample from each treatment without repetition using hedonic tests (level of preference) on semi-trained panelists. Determination of the formulation of adding *tempeh gembus* is through preliminary research. Salt, garlic, shallots, and pepper were used as flavoring agents. Egg white and baking soda were used as adhesives and dough developers.

Preliminary Treatment Samples

The main study used a completely randomized design of 1 factor, namely the substitution of *tempeh gembus* with 4 levels (25%, 50%, 75%, 100%) and 1 control (0% *tempeh gembus*) with 5 repetitions to obtain 25 experimental units for analysis of nutrient content and the level of acceptance.

The meatballs were formulated by weighing the ingredients according to the level of treatment. The *tempeh gembus* steamed and smoothed using a mortar. *Tempeh gembus* mixed with all mashed ingredients and ground meat. After that, shaping and boiling process is done in boiling water until the meatballs appear on the surface of the water ± 15 min.

Table 1: Formulation of Treatment in Research

Material	Amount of Material				
	0%	25%	50%	75%	100%
Minced beef	100 g	75 g	50 g	25 g	-
Tempeh Gembus	-	25 g	50 g	75 g	100 g
Tapioca flour	20 g	20 g	20 g	20 g	20 g
Pepper	2,5 g	2,5 g	2,5 g	2,5 g	2,5 g
Egg	20 g	20 g	20 g	20 g	20 g
Garlic	2 g	2 g	2 g	2 g	2 g
Red onion	2 g	2 g	2 g	2 g	2 g
Salt	1 g	1 g	1 g	1 g	1 g
Baking soda	1 g	1 g	1 g	1 g	1 g

Protein Content Analysis

The refined samples were weighed as much as 0.2 g and dissolved into the *Kjeldahl* flask. As much as 0.7 g of nitrogen catalyst which includes 250 g of Na_2SO_4 + 5 g of $CuSO_4$ + 0.7 g of Selenium / TiO_2 after that added 4 ml of concentrated H_2SO_4 is inserted to the flask through the flask wall. The solution was destroyed in the fume hood until the color of the solution turned clear green, then added 10 ml of aquadest. The solution was distilled by adding 20 ml of NaOH - TiO (NaOH 40% + $Na_2S_2O_3$ 5%) then the distillate was accommodated in Erlenmeyer which has been filled with 4% H_3BO_3 and the indicator Mr-BCG up to 60 ml where the color changes from red to blue. The distillate solution is tested using a standard 0.02 N HCl solution until it reaches the end point of the titration, which changes color from blue to pink and records the volume of the titration.

Calcium Contents Analysis

The samples were weighed as much as 500 mg. 25 mL of concentrated HCL added and heated it for 15 minutes. The sample was filtered in to a 50 mL measuring flask and distilled water to the limit.

The sample solution that was made was taken as much as 1 mL and neutralized with 40% NaOH. After neutral, the volume is sufficient to 10 mL. The neutral solution was taken as much as 1 mL and put it in a 25 mL measuring flask. After that, 1 mL of murexide solution and 2 mL of NaOH were added, then filled with distilled water to 25 mL. The solution is shaken until it is homogeneous and inserted into the cuvette to be read on the length of a wave of 534.6 μm.

Protein Digestibility Analysis

The samples weighed as much as 5 g were put into Erlenmeyer then added 20 ml of pH 2 whaffole buffer as much as 20 ml and 1% pepsin enzyme as much as 2 ml. The solution was incubated at 40°C for an hour. The solution was filtered or centrifuged and then 5 ml of 5% TCA was added. The solution was left to stand for an hour then 5 ml of filtrate was taken to analyze the protein content.

Acceptance Level Analysis

The organoleptic tests using hedonic tests (level of preference) on semi-trained panelists as many as 30 people from Nutrition Science students of Diponegoro University in the semester 4 and 6 grades with 5 preference scales, namely 1 = Very dislike, 2 = Dislike, 3 = Neutral, 4 = Like, 5 = Really Like. In this organoleptic test, there are 5 formulations in the form of 1 control formulation and 4 substitution formulations.

Statistical Analysis

All results of the experiments are expressed in median and mean ± standard deviation. Data were analyzed by using statistical software. Kruskal-Wallis and Friedman were used to test the mean differences and the statistical significance differences between mean values was established at $p < 0.05$. Mann-Whitney and Wilcoxon were used to post-hoc test.

Table 2: Results of Protein Content Analysis of *Tempeh Gembus* Meatballs

Protein Contents				
Addition of *Tempeh Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	5	11.27 (11.16-11.61)	11.30 ± 0.18^{a}	< 0.05
25%	5	8.04 (8.02-8.04)	8.03 ± 0.01^{b}	
50%	5	7.25 (7.19- 7.34)	7.26 ± 0.06^{c}	
75%	5	4.45 (4.40- 4.50)	4.45 ± 0.04^{d}	
100%	5	3.25 (3.08- 3.37)	3.23 ± 0.14^{e}	

*Kruskal-Wallis Test, Mann-Whitney Post-hoc Test; ** *Different superscript letters (a, b, c) show significant differences (p <0.05)*

Results

The results of the analysis of protein contents of *tempeh gembus* meatballs showed the difference between the addition of *tempeh gembus* in meatballs and protein contents ($p = 0.00$). Protein contents of all treatment groups are significantly different from controls and different between treatment groups. The highest protein content in the control class of *tempeh gembus* meatballs were 11.21%. The more additions of tempeh to the *tempeh gembus* meatballs, the protein content decreases significantly.

The results of the analysis of calcium content in *tempeh gembus* meatballs showed no difference between the addition of *tempeh gembus* in meatballs on calcium content ($p = 0.944$). Calcium contents across treatment groups did not differ significantly from controls. The highest calcium content is found in 100% *tempeh gembus* meatballs for 354.53 mg/100g.

Table 3 : Results of Calcium Analysis of *Tempeh Gembus* Meatballs

Calcium Contents				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	5	350.68 (343.35- 357.74)	351.44 ± 6.21	< 0.05
25%	5	351.45 (343.35- 356.84)	351.19 ± 5.36	
50%	5	354.40 (332.42- 356.20)	350.49 ± 10.13	
75%	5	354.53 (350.16- 356.20)	353.94 ± 2.29	
100%	5	355.30 (351.32-356.07)	354.53 ± 1.94	

* Kruskal-Wallis Test

The results of the analysis of protein digestibility of *tempeh gembus* meatballs showed the difference between adding *tempeh gembus* in meatballs and protein digestibility (p = 0.00). There is no significant difference between the treatment of 0% and 100%, 25% and 50%, 25%, and also 75%, and 50% and 75%. The highest protein digestibility in 100% *tempeh gembus* meatballs was 83.97%.

Table 4 : Results of Analysis of Protein Digestibility of *Tempeh Gembus* Meatballs

Protein Digestibility				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	5	83.71 (75.28- 89.21)	82.17 ± 5.37[a]	< 0.05
25%	5	54.06 (45.14- 60.15)	53.22 ± 5.52[b]	
50%	5	64.10 (47.82- 64.93)	58.44 ± 8.52[b]	
75%	5	56.22 (52.98- 75.92)	61.27 ± 10.13[b]	
100%	5	5 87.11 (71.15- 91.05)	83.96 ± 8.19[a]	

*Kruskal-Wallis Test, Mann-Whitney Post-hoc Test**;** **Different superscript letters (a, b, c) show significant differences (p <0.05)

Acceptance Level Test

Based on the statistical analysis with the Friedman test showing the addition of *tempeh gembus* in meatballs showed a significant increase in the color of meatballs (p = 0.00). The color of *tempeh gembus* meatballs with the substitution of 25% *tempeh gembus* had the highest level of acceptance of 3.80 (likes), while meatballs with 100% *tempeh gembus* content had the lowest level of acceptance of color of 2.73 (neutral).

Table 5: Results of Analysis of Acceptance Levels on the Color of Meatballs with *Tempeh Gembus* Substitution

Color				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	30	4.00 (2.00 - 5.00)	3.63 ± 1.03[a,b]	< 0.05
25%	30	4.00 (2.00 - 5.00)	3.80 ± 1.06[a]	
50%	30	3.00 (2.00 - 5.00)	3.16 ± 0.87[b]	
75%	30	3.00 (2.00- 5.00)	3.26 ± 0.78[b]	
100%	30	2.00 (2.00- 5.00)	2.73 ± 0.98[c]	

*Friedman Test, Wilcoxon Post-hoc Test; ** Different superscript letters (a, b, c) show significant differences (p <0.05)

The results of further test analysis showed that there were no significant differences in the treatment of 0% and 25%, 0% and 50%, 0% and 75%, 50%, and 75%.

Table 6: Results of Analysis of Acceptance Levels on the Taste of Meatballs with *Tempeh Gembus* Substitution

Taste				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	30	3.00 (2.00- 5.00)	3.23 ± 1.04	< 0.05
25%	30	3.00 (2.00- 5.00)	2.80 ± 0.80	
50%	30	3.00 (2.00- 5.00)	2.76 ± 0.89	
75%	30	3.00 (2.00- 5.00)	3.13 ± 1.22	
100%	30	2.00 (2.00- 5.00)	2.76 ± 0.97	

*Freidman Test

Table 7: Results of Analysis of Acceptance Levels on the Flavor of Meatballs with *Tempeh Gembus* Substitution

Flavor				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	30	3.00 (2.00- 5.00)	3.23 ± 1.04	< 0.05
25%	30	3.00 (2.00- 5.00)	2.80 ± 0.80	
50%	30	3.00 (2.00- 5.00)	2.76 ± 0.89	
75%	30	3.00 (2.00- 5.00)	3.13 ± 1.22	
100%	30	2.00 (2.00- 5.00)	2.76 ± 0.97	

*Friedman Test, Wilcoxon Post-hoc Test; ** Different superscript letters (a, b, c) show significant differences (p <0.05)

Based on statistical analysis with the Friedman test, the addition of *tempeh gembus* in meatballs did not significantly increase or decrease the meatball taste (p = 0.24). There was no significant difference in taste in the addition of *tempeh gembus* to the meatballs.

The analysis of the level of acceptance of meatball flavor with the addition of *tempeh gembus* showed significant results. There is a difference between the flavor of meatballs without the addition of *tempeh gembus* and with the addition of *tempeh gembus*.

The addition of *tempeh gembus* in meatballs significantly increased the level of acceptance of meatball flavor (p = 0.04). The most preferred meatballs flavor is meatballs with the addition of *tempeh gembus* by 25%, while meatballs with 50% addition have the lowest level of acceptance of the flavor which is equal to 3.03 (neutral).

The results of further test analysis showed that there were significant differences in the treatment of 0% and 50%.

Table 8: Results of Analysis of Acceptance Levels on the Texture of Meatballs with Tempeh Gembus Substitution

Texture				
Addition of *Tempehn Gembus* Levels	**n**	**Median (maximum- minimum)**	**Mean ± SD (%)**	**p***
0%	30	3.00 (2.00- 5.00)	$2.93 \pm 0.94^{a,b}$	< 0.05
25%	30	3.00 (2.00- 5.00)	3.16 ± 0.79^{a}	
50%	30	3.00 (2.00- 5.00)	$2.60 \pm 0.89^{b,d}$	
75%	30	3.00 (2.00- 5.00)	2.00 ± 0.87^{c}	
100%	30	2.00 (2.00- 5.00)	2.46 ± 1.13^{d}	

*Friedman Test, Wilcoxon Post-hoc Test; ** Different superscript letters (a, b, c) show significant differences (p <0.05)

The addition of *tempeh gembus* in meatballs gives a difference to the texture of meatballs (p = 0.00). The texture of meatballs with the addition of 25% *tempeh gembus* has the highest level of texture acceptance which is 3.16 (neutral). Whereas meatballs with the addition of 75% *tempeh gembus* have a low texture acceptance which is only 2.00 (dislike).

The results of further test analysis showed that there were no significant differences in the treatment of 0% and 25%, 0% and 50%, and 50% and 100%.

Discussion

Protein Contents

Addition of *tempeh gembus* in meatballs significantly reduced protein contents. In meatballs added with *tempeh gembus*, the protein content decreased due to the number of beef used and heat treatment during cooking. Meanwhile, previous study reported that the increase in temperature corresponded to the increase in protein denaturation and significant decrease in protein content as observed in the present study.[23] Protein contents in meatballs with *tempeh gembus* content of 25%, 50%, 75%, and 100%, differ significantly from the control. Meatballs with *tempeh gembus* content of 0% or controls have the highest protein content (11.21%), while meatballs without meat additives or 100% *tempeh gembus* have the lowest protein content (3.24%). However, when compared with meatball quality standards according to the National Standard of Indonesia, the meatball protein content with the formulation of 0% *tempeh gembus* or control has met these standards whic is minimum 11% protein.[24]

Meatball protein contents are influenced by protein content from raw materials. The increasing use of beef, it will show a tendency to increase contents of meatball protein produced. This is because beef has a higher protein content than *tempeh* *gembus*. The protein content of beef reaches 18.80 g per 100 g of ingredients,[25] Meanwhile, the protein content of *tempeh gembus* is only 6.7 g per 100 g of ingredients.[10]

In the results of the study, there was a decrease in protein contents in *tempeh gembus* meatballs when compared with the previous theory. This can be affected due to processing that occurs when making *tempeh gembus* and when making *tempeh gembus* meatballs. Making *tempeh gembus* through the fermentation process wherein the process is used *Rhizopus sp.* strain. The nitrogen compounds contained in the *tempeh gembus* protein are utilized by the mold of *Rhizopus sp.* for its growth.[26]

The steaming factor in *tempeh gembus* before making meatballs also affects the decrease of protein in meatballs. This was proven based on the research that had been done before, the *tempeh gembus* with steaming treatment had decreased in protein content compared to the *tempeh gembus* without any treatment.[27] Protein will be coagulated at a tempehrature of 100ºC and the water contained in the food will come out. This is because the protein loses its binding power to water so that the increase in steaming temperature will cause the protein to be dissolved and denatured.[28] In addition, the decrease in protein content in meatballs is also alleged to be the result of the addition of *tempeh gembus*, where the *tempeh gembus* contains a lot of fiber. Fiber can bind to water. Water can dissolve protein. The presence of fiber on meatballs due to the addition of *tempeh gembus* causes water to be unable to bind proteins perfectly because water binds to fiber so that a lot of protein is dissolved in the process of cooking meatballs.[29]

Calcium Contents

Calcium is the micronutrient needed by the body and the most abundant minerals in the body, which is 1.5-2% of an adult's body weight, or around 1,000-1,400 g per 70 kg of body weight.[30] Calcium analysis in this study used the Uv-Vis spectrophotometric method. This method is used for quantitative analysis of metal elements in trace amounts and ultra-trace amounts. This method gives the total amount of metal elements in a sample and does not depend on the molecular form of the metal in the sample.[31] Calcium contents in meatballs do not increase or decrease significantly. Calcium contents in the addition of *tempeh gembus* at 100% level with the highest calcium content of 354.53 mg/100 g of *tempeh gembus* meatballs. This result shows that the calcium content of the *tempeh gembus* meatballs is almost the same as beef meatballs. However, this is not in accordance with the theory of previous research. In a study conducted by Sulchan *et al.,* calcium contents contained in *tempeh gembus* were 143 mg /100g.[21] Whereas from the research conducted by Ruth *et al.,* that is equal to 232.09 mg/100g.[10]

Increased calcium contents are alleged due to the addition of ingredients found in *tempeh gembus* meatballs. In the process of making meatballs, added some ingredients such as 20% egg white, 20% tapioca flour, 2% onion, 2% garlic, 2,5% pepper, and 1% baking soda. Calcium content in egg white is 8 mg/100g,[32] tapioca flour 20 mg /100mg,[33] onion 36 mg /100 mg,[34] garlic 181 mg /100mg,[35] pepper 18 mg /100g,[36] baking soda 40 mg /100g.[37] Calcium content contained in the composer material of *tempeh gembus* can have an effect on the increase of calcium in *tempeh gembus* meatballs.

Protein Digestibility

Protein digestibility is the ability of proteins to be hydrolyzed into amino acids by digestive enzymes; which if the protein digestibility is high means that proteins can be hydrolyzed properly into amino acids so that the number of amino acids that can be absorbed and used by the body is high, while low protein digestibility means proteins are difficult to hydrolyze into amino acids so that the amount of amino acids that can be absorbed and is used by a low body because most of it will be removed by the body together with feces.[38]

Addition of *tempeh gembus* in meatballs had significant effect on digestibility of meatball protein ($p = 0.00$). The highest protein digestibility is found in meatballs with 100% *tempeh gembus* content of 83.97%. This result shows a high number. Protein digestibility is high if the digestive power is equal to or greater than 80%.[39] While the lowest digestibility of the protein is found in meatballs with the *tempeh gembus* content of 25% and 50%. This is not in accordance with previous research. In the previous study, it was stated that the *tempeh gembus* with the steaming process had a protein digestibility of 48.68%.[27] This was alleged because, at the time of making the *tempeh gembus* meatballs, meatballs underwent a boiling stage. The boiling stage can make composed proteins become denatured. Denaturation is the breaking of bonds in molecules so that protein molecules will tend to be easily digested by digestive enzymes.[28] Protein that has been denatured will be easily digested.[38]

The digestibility of *tempeh gembus* meatball protein in the treatment of 25%, 50%, and 75%, decreased in value compared to meatballs with a treatment *tempeh gembus* of 0% and 100%. This is alleged due to the formation of cross-linking proteins. Cross-linking that occurs causes proteases such as trypsin to find difficulty in breaking or breaking peptide bonds in cross-linked proteins. Lysine will easily interact with the aliphatic carbon group [- $(CH_2)_4$-NH_3] so that the availability of lysine becomes less useful.[40]

Acceptance Level

Color

In organoleptic tests, at first, the product is assessed visually by looking at the colors they have. Color is the first sensory that can be seen directly by panelists. Determination of the quality of food generally depends on the color it has. Color that does not deviate from the color that should be, will give the panelists a distinctive impression.[41]

The addition of *tempeh gembus* to the meatball had a significant effect on the level of acceptance of meatball color (p = 0.00). The average rating value of the panelist's hedonic test on meatball color ranges from 2.73 to 3.80 (neutral-like). Panelists generally like the color of meatballs from all treatments caused by the color of meatballs in this study the same as the color of beef meatballs on the market. However, on meatballs with a content of 100% *tempeh gembus* without the addition of meat, the resulting color is slightly paler compared to meatballs with a mixture of meat.

The color of grey brownish meatballs comes from the process of heating or boiling meatballs dough. During heating, the color of the meat will gradually change from pink to paler. The color change is a result of the amount of myoglobin pigment being oxidized to metmyoglobin and protein polymerization.[42] In making meatballs, meat should be used in the pre-rigormortis phase. This is because the use of meat in this phase affects the color of the meatballs produced. The use of post-rigor beef will produce a whiter meatball color when compared to pre-rigor meat.[43]

Taste

Taste is a determining factor for consumer acceptance of food products. Formulations of spices, composer and meat conditions for making meatballs greatly affect the taste of the meatballs produced.[42] The amount of *tempeh gembus* added to the meatballs did not affect the level of acceptance of the *tempeh gembus* meatballs (p = 0.24). The average value of the panelist assessment of the hedonic test for meatball taste ranges from 2.76 to 3.23 (neutral). The 0% treatment of *tempeh gembus* or 100% beef meatballs received the highest score of 3.23 which indicates that the panelists prefer meatballs with a content of 100% beef because it is more savory.

The savory typical value of meatballs is obtained from glutamic acid contained in beef.[25] Glutamic acid contained in beef reaches 14.4 g/100g of ingredients. Whereas, glutamic acid contained in the *tempeh gembus* only 0.29%/100g *tempeh gembus* wet weight.[21] Besides glutamic acid, meatballs from pre-rigor meat also have a better taste. This is because pre-rigor meat has a water binding capacity and high pH which increases tenderness and juicy in meat.[44]

Flavor

The assessment of the flavor of food is an evaluation of the sense of smell. Addition of *tempeh gembus* to the meatball had a significant effect on the level of acceptance of meatball flavor (p = 0.04). The average value of the hedonic test by the panelists on meatball flavor ranges from 3.03 to 3.70 (neutral-like). The highest value (most preferred) for the flavor assessment was obtained from the chopped *tempeh gembus* meatballs with the *tempeh gembus* formulations of 0%. Spices such as garlic can enhance and modify the flavor. Spices are material that is intentionally added to improve consistency, nutritional value, taste, control acidity, and basicity, and to strengthen the shape and appearance of products.[42] Spices are also useful for increasing taste, as an antioxidant that can reduce rancidity, and as an anti-microbial that can extend the shelf life of meatballs. In addition, based on research on soybean tempeh, the fat contained in soybean tempeh is resistant to the rancidity process which is influenced by the production of natural antioxidants by mold tempeh.[45]

Texture

Texture assessment is done to find out whether the surface of the *tempeh gembus* is elastic. Elasticity is the ability of food to return to its original form after being pressured.[33] The addition of *tempeh gembus* to the meatballs has a significant effect on the level of acceptance of meatball texture ($p = 0.00$). The average value of the assessment level of the hedonic test by the panelists on the texture of meatballs ranges from 2.00 to 3.16 (dislike-neutral). The highest value (most preferred) for the texture assessment was obtained from the *tempeh gembus* meatballs with the *tempeh gembus* formulations of 25%. Whereas, the smallest value (disliked and highly disliked) was obtained on the *tempeh gembus* meatballs with the *tempeh gembus* formulations of 100% and 75%. Factors that influence the tenderness value are connective tissue and marbling fat contained in the product, as well as the temperature which has an influence on the binding capacity of water by meat protein, cooking shrinkage, pH and meat juice content.[44]

The elasticity of 25% meatballs is alleged due to the high binding capacity of water from meat. Beef has 66% moisture content per 100g of ingredients. Whereas, the steaming *tempeh gembus* has a water content of 65.22%.[27] The binding capacity of water can be defined as the ability of the meat to maintain its water content during external treatments such as cutting, heating, grinding, and processing. Protein contents also become an influencer, because the higher the protein content, the higher the water bound, thus the meatballs will be more elastic.[42]

Determination of Selected Formula[39]

The best products are selected by giving weighting to the test results on *tempeh gembus* meatballs. The first thing to do is to give weight to the results of the hedonic test conducted by Mansyhur (2017). Weighting is given to four indicators which are scored corresponding to their interests according to the panelists. The taste and flavor are given the highest weighting, which is 40% because taste and flavor are the most important indicators in determining the first acceptance of a product.[43] Furthermore, the color and texture are given a weight of 10%.

Table 9: Hedonic Test Attribute Weighting Results

Indicators	0%	25%	50%	75%	100%
Color	1.48	1.4	1.21	1.39	1.37
Taste	1.29	1.12	1.21	1.25	1.11
Flavor	1.48	1.4	1.21	1.39	1.37
Texture	0.29	0.32	0.26	0.2	0.25
Over all	4.54	4.24	3.79	4.23	4.10

The first rank is obtained by a hedonic test with a control treatment or 0%, while the second rank is obtained by a hedonic test with a treatment of 25%. Because in this study the researchers sought the best substitution formulation, the researchers took the second rank with 25% treatment.

The next step after weighting the results of the hedonic test is weighting of each formula according to the results of the test of protein content, calcium content, and protein digestibility. The formula that has the highest value of the test done will get the highest score.

Table 10: Selected Formulation Determination Scores

Formula	Hedonic Test (40%)	Score	Protein Content (20%)	Score	Calcium Content (20%)	Score	Protein Digestibility (20%)	Score	Score (100%)	Scoring Result
0%	4	1.6	5	1	3	0.6	4	0.8	4	
25%	5	2	4	0.8	2	0.4	1	0.2	3.4	Selected Formula
50%	1	0.4	3	0.6	1	0.2	2	0.4	1.6	
75%	3	1.2	2	0.4	4	0.8	3	0.6	3	
100%	2	0.8	1	0.2	5	1	5	1	3	

The formulation with the highest value is the treatment of 0% and 25%. However, because the researchers wanted to find the best treatment for the substitution of *tempeh gembus*, the treatment of meatballs with the substitution of 25% *tempeh gembus* can be used as an alternative in making local food-based snacks. Meatballs formulation of 25% has a protein content of 8.03%, calcium content 351.19 mg/100g, and protein digestibility of 53.22%.

The results of the hedonic quality assessment show that meatball formulation of 25% has characteristics such as gray which is favored by panelists (3.80), taste that is considered neutral by panelists (2.80), meatball flavor preferred by panelists (3.50), and a little compact texture that is considered neutral by panelists (3.17).

Furthermore, the results of the analysis of protein and calcium content from the formulation were 25% compared to the Nutritional Label Reference (NLR). NLR for the general category according to PKBPOM Number 9 of 2016 concerning Reference to Nutritional Label Reference is 60 g protein and 1100 mg for calcium.[46] Below is a comparison of the results of the analysis of % NLR

Table 11: Energy Content, Protein, Calcium per Serving Dose

Components	Analysis Result	% NLR (%)
Protein (g/100g)	8.03	13.4
Calcium (mg/100g)	351.19	31.9

Analysis of the Contribution of Nutritional Content to DV and NRL

The serving dose of the *tempeh gembus* meatball products is determined based on the number of the daily value (DV) in adolescents 16-18 years. The serving dose is determined to assume the meatballs are consumed as a snack with a minimum percentage of 10%.

Table 12 : Contribution of Nutritional Content to DV and NRL

Components	Nutritional Content (per serving 78 g)	% DV in Adolescents 16-18 Years		% NLR
		Boys 16-18 Years	Girls 16-18 Years	
Energy (kkal)	270	10%	13%	13%
Protein (g)	6.3	10%	11%	11%
Calcium (mg)	273.9	23%	23%	25%

From the results above, it can be seen that the fulfillment of the DV and NLR from 25% *tempeh gembus* meatballs is only about 10-20% of the daily requirement. Therefore, other additional foods are needed to meet the needs of calcium and protein for body needs in a day.

Conclusion

The interaction of substitution of *tempeh gembus* with meat content in *tempeh gembus* meatballs has an effect on protein contents and protein digestibility but had no effect on increasing calcium meatball contents for body needs in a day.

Based on an analysis of nutrient content, protein digestibility, and organoleptic tests formulation with 25% *tempeh gembus* substitution was found to be a best formulation of meatballs containing protein content of 8.03%, calcium contents of 351.19 mg / 100 g, and protein digestibility of 53.22%. It is therefore important that advances in development of *tempeh gembus* combination that has significant value in increased the calcium content in addition to the increase the protein content, protein digestibility and acceptability of *tempeh gembus* meatballs.

Suggestions

Need to do further research to increase the level of preference for the taste of *tempeh gembus* meatballs.

Acknowledgment

The authors are thankful to the Research Institute of Diponegoro University which facilitated and funded this research.

Funding

The author(s) received financial support for the research, authorship, and/or publication of this article from Research Institute of Diponegoro University with number of grant: 474-71/UN7.P4.3/PP/2018.

Conflict of Interest

The authors do not have any conflict of interest.

References

1. Oleson C.V., Morina A. B. Osteoporosis Rehabilitation: A Practical Approach. Springer; 2017. 5 p. https://doi.org/10.1007/978-3-319-45084-1
2. Kementerian Kesehatan RI. Data dan Kondisi Penyakit Osteoporosis di Indonesia. Jakarta; 2015.
3. Nafilah, Fitranti D.Y. Hubungan Indeks Massa Tubuh (IMT), Persen Lemak Tubuh, Asupan Zat Gizi, dan Aktifitas Fisik dengan Kepadatan Tulang pada Remaja Putri. *Nutrition College*. 2014;3(4):680–688.
4. Faizah L. N., Fitranti D. Y. Hubungan Asupan Protein, Fosfor, dan Kalsium dengan Kepadatan Tulang pada Wanita Dewasa Awal. *Nutrition College*. 2015;4(2):335–341.
5. Fikawati S., Syafiq A., Puspasari P. Faktor-Faktor yang Berhubungan dengan Asupan Kalsium pada Remaja di Kota Bandung. *Jurnal Kedokteran Trisakti Universa Medicina.* 2005;24(1):24-34.
6. Mahan L. Kathleen., and Sylvia Escott-Stump. Krause's Food & Nutrition Therapy. 12th ed. Canada: *Saunders Elsevier*; 2008.
7. Harold N Rosen, MD. Patient education: Calcium and Vitamin D for Bone Health (Beyond the Basics). 2015. Available from: http://www.uptodate.com/contents/calcium-and-vitamin-d-for-bone-health-beyond-the-basics
8. Damardjati D., Marwoto D.K., Swastika D., Arsyad, Hilman Y. Prospek dan Arah Pengembangan Agribisnis Kedelai. Jakarta; 2005.
9. Rahmawati Widi Deshinta. Pengaruh Substitusi Tepung Ampas Tahu terhadap Tingkat Kekerasan dan Daya Terima Bakso. [Skripsi]. Surakarta: Universitas Muhammadiyah Surakarta; 2016.
10. Damanik R. N. S., Pratiwi D. Y. W., Widyastuti N, Rustanti N, Anjani G, Afifah D. N. Nutritional Composition Changes during *Tempeh gembus Processing. IOP Conf Ser Earth Environ Sci*. 2018;116(1). doi:10.1088/1755-1315/116/1/012026
11. Murdiati, A., Sardjono, Amaliah. Perubahan Komposisi Kimia Tempe Gembus yang Dibuat dari Bahan Dasar Ampas Tahu dan Bekatul. *Agritech.* 2002;20(2):106–110.
12. Afifah D. N., Nugrahani G., Hastuti V. N., Arifan

F. The Characteristics of Kerupuk Gembus The Characteristics of Kerupuk Gembus. *IOP Conf Ser Earth Environ Sci*. 2019;292(1). doi:10.1088/1755-1315/292/1/012055

13. Noviana A., Dieny F. F., Rustanti N., Anjani G., Afifah D. N. Antimicrobial Activity of *Tempeh gembus* Hydrolyzate. *IOP Conf Ser Earth Environ Sci*. 2018;116(1). doi:10.1088/1755-1315/116/1/012044
14. Kurniasari R, Sulchan M, Afifah DN, Anjani G, Rustanti N. Influence Variation of Tempe Gembus (an Indonesian Fermented Food) on Homocysteine and Malondialdehyde of Rats fed an Atherogenic Diet. *Rom J Diabetes, Nutr Metab Dis*. 2017;24(3): 203-211. doi:10.1515/rjdnmd-2017-0026
15. Afifah D. N., Sulchan M., Syah D., Yanti, Suhartono M. T., Kim J. H. Purification and Characterization of A Fibrinolytic Enzyme from Bacillus pumilus 2.g isolated from gembus, an Indonesian fermented food. *Prev Nutr Food Sci*. 2014;19(3):213-219. doi:10.3746/pnf.2014.19.3.213
16. Afifah D. N., Sulchan M., Syah D., Yanti., Suhartono M. T. Isolation and Identification of Fibrinolytic Protease-Producing Microorganisms from Red Oncom and Gembus, Indonesian Fermented Soybean Cakes. *Malaysian Journal of Microbiology*. 2014;10(4):273-279.
17. Afifah D. N., Rustanti N., Anjani G., Syah D., Yanti., Suhartono M. T. Proteomics Study of Extracellular Fibrinolytic Proteases from Bacillus licheniformis RO3 and Bacillus pumilus 2.g isolated from Indonesian Fermented Food. *IOP Conf Ser Earth Environ* Sci. 2017;55:1-10.
18. Dewi P. K., Afifah D. N., Rustanti N., Sulchan M., Anjani G. The Effect of *Tempeh gembus* Variations to Serum Levels of High Sensitivity C-Reactive (hsCRP) and Serum Levels of Fibrinogen of Sparague Dawley Rats with Atherogenic Diet. *Rom J Diabetes, Nutr Metab Dis.* 2018;25(1):91-97. doi: 10.2478/rjdnmd-2018-0010.
19. Afifah D. N., Nabilah N., Supraba G. T., Pratiwi S. N., Nuryanto., Sulchan M. The Effect of *Tempeh gembus*, an Indonesian Fermented Food, on Lipid Profiles in Women with Hyperlipidemia. *Current Nutrition & Food Science.* 2018;14(1):1-9. doi:10.2174/1573401314666180807112549
20. Agustina R. K., Dieny F. F., Rustanti N., Anjani G., Afifah D. N. Antioxidant activity and soluble protein content of *tempeh gembus hydrolysate. Hiroshima J Med Sc*i. 2018;67:1-7.
21. Sulchan M., N.W, Endang. Nilai Gizi dan Komposisi Asam Amino Tempe Gembus serta Pengaruhnya terhadap Pertumbuhan Tikus. *Maj Kedokt.* 2007;57(3):80–85.
22. Bastian F., Ishak, Tawali, Bilang M. Daya Terima dan Kandungan Zat Gizi Formula Tepung Tempe dengan Penambahan Semi Refined Carrageenan (SRC) dan Bubuk Kakao. *Aplikasi Teknologi Pangan*. 2013;2.
23. Alakali JS, Kucha CT, Rabiu IA. Effect of drying temperature on the nutritional quality of Moringa oleifera leaves. *African J Food Sci.* 2015;9(7):395–9.
24. Badan Standar Nasional. Standar Nasional Indonesia: Bakso Daging. Jakarta: BSN; 014. 2-3 p.
25. Purwanto A., Ali A., Herawati N. Kajian Mutu Gizi Bakso Berbasis Daging Sapi dan Jamur Merang *(Volvariella volvaceae). Teknologi Hasil Pertanian.* 2015;14(2):1–8.
26. Sayudi S., Herawati N., Ali A. Potensi Biji Lamtoro Gung dan Biji Kedelai sebagai Bahan Baku Pembuatan Tempe Komplementasi. *Jom Faperta.* 2015;1(2):1–9.
27. Afifah D.N., Rahma A.,Nuryandari S.S., Alviche L., Hartono P.I., Kurniawati D.M., Wijayanti H.S., Fitranti D.Y., Purwanti R. Nutrition Content, Protein Quality, and Antioxidant Activity of Various Tempeh Gembus Preparations. *Journal of Food and Nutrition Research.* 2019; 7(8):605-612. doi:10.12691/jfnr-7-8-8.
28. Sumiati T. Pengaruh Pengolahan terhadap Mutu Cerna Protein Ikan Mujair (*Tilapia mossambica*). [Skripsi]. Bogor: Institut Pertanian Bogor; 2008.
29. Takarina H. L. Kandungan Kalsium dan Karbohidrat Bakso Daging Sapi dengan Penambahan Jamur Tiram (Pleurotus sp). [Skripsi]. Surakarta: Universitas Muhammadiyah Surakarta; 2013.
30. Gropper S. S., Smith J. L., Groff J. L. Advanced Nutrition and Human Metabolism. 5th ed.

USA: Cengage Learning; 2009. 431 p.
31. Gandjar I. G., Rahman A. Kimia Farmasi Analisis. Yogyakarta: Pustaka Pelajar; 2007. p. 240–62, 463–74.
32. Muchtadi T. R., Sugiyono, Ayustaningwarno F. Ilmu Pengetahuan Bahan Pangan. Bandung: Alfabeta; 2010.
33. Soemarno. Rancangan Teknologi Proses Pengolahan Tapioka dan Produk-Produknya. Malang: Universitas Brawijaya; 2007.
34. Wibowo S. Budidaya Bawang Merah, Bawang Putih, Bawang Bombay. Jakarta: Penebar Swadaya; 2008.
35. USDA. National Nutrient Database for Standard Reference of Raw Garlic [Internet]. 2010. Available from: https://ndb.nal.usda.gov/ndb/foods/show/301861
36. USDA. National Nutrient Database for Standard Reference of Raw Pepper [Internet]. 2018. Available from: https://ndb.nal.usda.gov/ndb/foods/show/302197
37. USDA. National Nutrient Database for Standard Reference of Raw Baking Soda [Internet]. 2018. Available from: https://ndb.nal.usda.gov/ndb/foods/show/45016339
38. Saputra D. Penentuan Daya Cerna Protein In Vitro Ikan Bawal (Colossoma macropomum) pada Umur Panen Berbeda. *Food Technology.* 2014;5(2):1127–1133.
39. Hanifah., Kusharto C. M. Daya Cerna Protein Serta Kandungan Mineral (Kalsium dan Fosfor) pada Snack-Bar Substitusi Tepung Pisang (*Musa paradisiaca*) dengan Tepung Pury Ulat Sutera (*Bombyx mori*) dan Tepung Lele (Clarias gariepinus). [Skripsi]. Bogor: Institut Pertanian Bogor; 2017.
40. Budiman., Wulandari Z., Suryati T. Suplementasi Tepung Putih Telur untuk Memperbaiki Nilai Nutrisi Snack Ekstrusi Berbahan Grits Jagung. *Media Peternak.* 2009;32(3):179–184.
41. Negara J. Aspek Mikrobiologis serta Sensori (Rasa, Warna, Tekstur, Aroma) pada Dua Bentuk Penyajian Keju yang Berbeda. *Ilmu Produksi dan Teknologi Hasil Peternakan.* 2016;4(2):286–290.
42. Firahmi N., Dharmawati S., Aldrin M. Sifat Fisik dan Organoleptik Bakso yang Dibuat dari Daging Sapi dangan Lama Pelayuan Berbeda. *Sains dan Teknologi.* 2015.
43. Hatta M. Pengaruh Level dan Waktu Penambahan Fosfat (Sodium Tripolifosfat/STTP) terhadap Kualitas Bakso. *Jurnal Agrisistem*. 2011;7:87–95.
44. Soeparno. Ilmu dan Teknologi Daging. Yogyakarta: Gadjah Mada University Press; 2009.
45. Murni M. The Effect of Addition Tempeh Flour to The Quality and the Taste of Chicken Nugget. *Berita Litbang Industri.* 2014;3(2):117–23.
46. PKBPOM. Acuan Label Gizi. Jakarta; 2016.