## ORIGINAL ARTICLE

# Effect of Addition Tempeh Flour on Calcium, Protein, Water Content and Acceptance in Catfish Meatballs

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### ABSTRACT

**Introduction:** More than 50% of women in each country suffer from dysmenorrhea. This can be painful during menstruation and affect 50% of a woman's daily activities. Calcium can reduce muscle cramps after contractions. Tempeh and catfish are calcium-rich foods that people often eat, but the variety of processed products available is limited. The purpose of this study was to analyze the feasibility and compatibility of catfish meatballs with tempeh flour added with SNI fish meatballs as a healthy diet food for reducing dysmenorrhea in adolescents. **Method:** The research design applied was true experimental with 12 experimental units. The calcium content of the samples was determined using Atomic Absorption Spectrophotometry (AAS), the protein content used the Kjeldahl technique, the water content used the Oven method, and the acceptance test used the Hedonic Scale Test on adolescent. Data were analyzed using the One-Way ANOVA, and the acceptance test results were analyzed using the Friedman test with significance level  $\alpha$ =0.05. The proportion of tempeh flour on catfish meatballs was 0% (P0), 10%, 20% and 30% (P10, P20, and P30). **Results:** The results showed that the adding tempeh flour could improve the levels of calcium, protein, and acceptability (taste) of catfish meatballs. The addition of 10 percent (P10) tempeh flour was an acceptable composition of the taste, but its value did not differ significantly from other recipes. **Conclusion:** Catfish meatballs P30 has the highest calcium and protein content. Further research is needed to fine-tune the flavor and confirm its effectiveness in reducing menstrual cramps.

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#### **INTRODUCTION**

Calcium, iron and iodine are nutrients that are generally deficient in food, especially in developing countries (1). Calcium deficiency in children and adolescents results in growth problems; the hardening of the bones becomes obstructed and causes rickets (2). Calcium is not only needed for bone growth; for young women, calcium is also needed to reduce or even avoid pain before or during menstruation or what is commonly known as dysmenorrhea. According to Hidayati et al, (3) calcium intake is related to the incidence of dysmenorrhea in adolescent girls where the higher the calcium intake, the less pain is felt, or the young women do not feel pain. Meatballs are one of the most popular alternative foods in the community besides noodles (4). Meatball making does not have to use beef as the main ingredient because the price of beef tends to be expensive. Meatball modification can take advantage of catfish meat, which is easily found in every region. Based on data from the Livestock Service Office and Fisheries Service Office of Jember Regency, catfish production was in the 3rd highest position in 2016 after broilers and broiler eggs amounted to 6,965.90 tons. This shows that the public consumption of catfish is high. The calcium content in catfish is also relatively high, 289mg per 100g and protein 18.7g per 100g, while the calcium content in chicken is 14mg per 100g and protein 18.2g per 100g (5).

The ingredient needed for meatballs is tapioca flour, which has only a tiny amount of calcium. One of the alternative sources of calcium as a substitute for tapioca flour is tempeh flour. Changing its shape to flour makes wider use of tempeh and enables a long shelf life(6). The nutritional content of tempeh is also classified as high, 20.8g protein per 100g and calcium content of 155mg per 100g (5). The high calcium content in catfish and tempef flour makes them a good source of calcium.

The addition of tempeh flour is also expected to enrich the calcium content in catfish meatballs to be used as an alternative menu to replace rice to reduce the risk of dysmenorrhea for women from an early age. According to Widodo et al. (2013), (7) the average adequacy of energy, vitamin A, folic acid, vitamin C, calcium and iron is still below the RDA. Based on the characteristics of the age group, the lowest level of calcium consumption occurred in the age group of 9-12 years is as much as 47%. Meanwhile, based on gender characteristics, the consumption level of women is lower than that of men, which is 73.1%. Consumption of polyunsaturated fatty acids (omega-3 fatty acids) can help alleviate pain associated with dysmenorrhea (8). Fish is a source of omega-3 fatty acids, one of which is catfish (8). Low consumption of fish is associated with the severity of dysmenorrhea (9). The purpose of this study was to analyze the effect of adding 10%, 20% and 30% of Tempeh flour on calcium, protein, water content and acceptability of catfish meatballs and their confirmity with SNI for fish meatballs.

### MATERIALS AND METHODS

There were four levels of research treatment: the first level is P0 (without adding tempeh flour/control), the second level is P10 (adding 10 per cent tempeh flour), third level is P20 (adding tempeh flour 20 per cent), and the rest is P30 (adding tempeh flour 30 per cent) with replication (repetition) 3 times, so that the total sample was 12 experimental units (Table I). The calcium content of the samples was determined using Atomic Absorption Spectrophotometry (AAS), the protein content was determined using the Kjeldahl technique, and the water content was determined using the Oven method.

 
 Table I: The proportion of tapioca flour and Tempe flour in the Catfish meatball composition at 4 treatment levels

	Catfish meat	Tapioca flour	Weight	Tempe flour	Weight
P0	500 g	100 percent	250 g	0 percent	0 g
P10	500 g	90 percent	225 g	10 percent	25 g
P20	500 g	80 percent	200 g	20 percent	50 g
P30	500 g	70 percent	175 g	30 percent	75 g

AAS gives the total concentration of metal elements in a sample and does not depend on the molecular shape of the metal in the sampel. This method is suitable for trace metal analysis because it can determine metal levels with high sensitivity (detection limit with very small concentrations, which are less than 1 ppm) (10). The Kjeldahl method is the simplest method of determining total nitrogen for amino acids, proteins and compounds with nitrogen. The sample was melted with H2SO4 and catalyzed using an appropriate catalyst so as to produce (NH4)2SO4. The ammonia that has been liberated using strong alkali is steam distilled in an absorbent solution and then titrated. This method is suitable if made semi-micro since the sample and reagents required are not much and the analysis time is short (11). The oven method

is the sample was dried in an air oven at a temperature of 100-105°C until a stable weight was obtained (12). The test was carried out at the Food Analysis Laboratory of the Food Industry Technology Study Program, Jember State Polytechnic.

The acceptance test used the Hedonic Scale Test based on the liking scale (5 = really liked, 4 = liked, 3 = ordinary, 2 = disliked, 1 = very disliked). The acceptance test was conducted at Primary School Muhammadiyah 1 Jember on 25 menstruating female students aged 9-12 years (untrained panelists) that had met the minimal requirement for untrained panelists for a hedonic test (13). Researchers chose the age range of 9-12 years because it is the age of starting menstruation with a tendency to intake that dose not meet the RDA for calcium and protein (7).

The inclusion criteria were willingness to be a sample, being in the place when the research was conducted, and had entered the mentrual period. Meanwhile, the exclucion criteria were having taboo or restrictions or alergies to food ingredients, being in a state of illness that could cause limited food to be consumed, including the product to be tested, and the product being tested was the panelist's favorite food or disliked food.

The independent variable in this study is the addition of tempeh flour, while the dependent variable is calcium, protein, water content and acceptability. Sources of data used in this study are primary data (observational data and laboratory data). The Friedman and Wilcoxon Signed Ranks tests were used to examine the acceptance test results. Calcium, protein, and water content were determined using the One Way Anova and Post Hoc Test (Bonferroni) with a 5% (= 0.05) significant level. These research has ethical clearance number 1303/UN.25/KEPK/DL/2017.

## RESULTS

## Levels of Calcium Catfish Meatballs

According to the findings of laboratory calcium analysis (Table II), catfish meatballs with tempeh flour added at P0, P10, P20, and P30 has enhanced calcium levels. It can be seen from the graph, the calcium value of each treatment for adding tempeh flour is 19.22 m/100g (P0); 36.7 mg/100g (P10); 51.01 mg/100g (P20) and the highest was 63.26 mg/100g (P30). In the One-Way ANOVA test, a significance value of 0.000 ( $\leq$  0.05) was obtained. It means that there is a significant difference from the addition of tempeh flour with various treatments on the calcium content of catfish meatballs. All samples in Post Hoc test analysis showed significant differences with p value (0.000) les than 0.05.

### **Catfish Meatballs Protein Content**

According to the protein content analysis (Table II), the protein contents of catfish meatballs with or without

Table II: Calcium, Protein, and Water Content in Catfish Meatballs

	P0	P10	P20	P30	P value**
Calcium	19.22	36.70	51.01	63.26	0.000
Protein	17.60	21.61	24.79	27.61	0.000
Water	47.66	46.49	46.18	45.70	0.000

\*Data represented on a wet basis

\*\*ANOVA significantly different (p-value ≤0.05)

tempeh flour at four treatment levels (P0, P10, P20, and P30) vary from 17.60% to 21.61%; 24.79% to 27.61%. It can be seen in the graph that along with the addition of tempeh flour, the protein value of catfish meatballs also increases. In the One-Way ANOVA test, a significance value of 0.000 ( $\leq$  0.05) was obtained, which means that there is a significant difference from the addition of tempeh flour with various treatments on the protein content of catfish meatballs. All samples in Post Hoc test analysis showed significant differences with p value (0.000) les than 0.05.

#### Water Content of Catfish Meatballs

According to the water content, catfish meatballs with tempeh flour added at P0, P10, P20, and P30 show a reduced water content. It can be seen from the results that the average value of each treatment for adding tempeh flour is 47.66% (P0); 46.49% (P10); 46.18% (P20), and the lowest average value of 45.70% (P30). In the One-Way ANOVA test, a significance value of 0.000 ( $\leq$  0.05) was obtained; it means that there is a significant difference from the addition of tempeh flour with various treatments on the water content of catfish meatballs. All samples in Post Hoc test analysis showed significant differences with p value (0.000) les than 0.05.

#### Addition of Tempe Flour to the Acceptability of Catfish Meatballs (Taste, Color, Aroma and Texture)

According to the Hedonic Scale Test findings, the panelists found that the flavor of catfish meatballs without or with tempeh flour is satisfactory (Figure 1). The treatment with the highest value is the treatment with the addition of 10 % tempeh flour (P10) with a value of 3.56 (ordinary-like). In comparison, the lowest value is the treatment with the addition of 20% tempeh flour (P20) with a value of 2.52 (dislike - ordinary). According to the analysis results using the Friedman test, the acceptance of the taste of catfish meatballs has a significance level of 0.000, so the conclusion is that H0 is rejected (there is a significant difference from the addition of tempeh flour to the taste acceptance of catfish meatballs). The Wilcoxon Signed Ranks Test analysis in Table 4 shows that the acceptability of the taste of catfish meatballs showed p-value < (0.05)with significantly different treatments, namely P0-P20 P10-P20, P10-P30 and P20-P30.



Figure 1: The average value of acceptance of taste, color, aroma, and texture of catfish meatballs without and with the addition of tempeh flour 0% (P0), 10% (P10), 20% (P20) and 30% (P30).

Table III: The p-value of Friedman Test for Acceptability of Catfish-Meatballs

The	The p-				
	value of FriedmanTest				
	Taste <sup>a</sup>	$Color^{b}$	Aroma <sup>c</sup>	Texture <sup>d</sup>	
P0 (0%)	3.28	3.4	3.4	3.16	0.000*a
P10 (10%)	3.56	3.6	3.76	3.48	0.456 <sup>b</sup>
P20 (20%)	2.52	3.52	3.36	3.16	0.116 <sup>c</sup>
P30 (30%)	3.08	3.36	3.44	3.24	0.203 <sup>d</sup>

\*the mean is significantly different (p-value ≤0.05)

According to the Hedonic Scale Test findings, the color of catfish meatballs without or with tempeh flour is deemed satisfactory by the judges (Figure 1). The treatment with the highest value is the treatment with the addition of 10% tempeh flour (P10) with a value of 3.6 (ordinary - like). In comparison, the lowest value is the treatment with the addition of tempeh flour 30% (P30) with a value of 3.36 (ordinary-like). The analysis results using the Friedman test indicate that the acceptability of the color of catfish meatballs has a significance level of 0.456 so that H0 is accepted (the level of preference for color parameters in the addition of catfish meatball tempeh flour is not significantly different).

Based on the Hedonic Scale Test results, the aroma of catfish meatballs without or with the addition of tempeh flour was acceptable to the panelists. The treatment with the highest value was the treatment with the addition of 10% tempeh flour (P10) with a value of 3.76 (normal-like). In comparison, the lowest value is the treatment with the addition of 20% tempeh flour (P20) with a value of 3.36 (ordinary - like). According to the Friedman test results, the acceptance of the scent of catfish meatballs has a significance level of 0.116, indicating that H0 is accepted (the degree of liking for fragrance criteria is not substantially different when catfish meatball tempeh flour is added).

According to the findings of the Hedonic Scale Test, the texture of catfish meatballs without or with tempeh flour is deemed satisfactory by the panelists (Table III). The treatment with the highest value was the treatment with the addition of 10% tempeh flour (P10) with a value of 3.48 (ordinary-like). In comparison, the lowest value is the treatment with the addition of 20% tempeh flour (P20) with a value of 3.16 (ordinary - like). The analysis results using the Friedman test show that the acceptance of the texture of catfish meatballs has a significance level of 0.203, so the conclusion is that H0 is accepted (the level of preference for texture parameters in the addition of catfish meatball tempeh flour is not significantly different).

## DISCUSSION

Based on the laboratory analysis results, it is known that the highest calcium content in catfish meatballs is the addition of tempeh flour as much as 30% (P30), and the lowest calcium content is in the catfish meatball treatment without adding with tempeh flour (P0). The increased calcium content of catfish meatballs is owing to tempeh's greater calcium content than catfish; hence, the calcium content rises when tempeh flour is added to catfish meatballs. This is also supported by study Suhartini, et.al (14); when Moringa leaf flour (moringa oleifera) is added to tempeh recipe biscuits, the protein and calcium content increases by 9%. Additionally, other research on tempeh flour substitution in noodles notes a rise in calcium levels.(14). Another study (15) found that tempeh flour and fish meal had a substantial influence on the protein, calcium, flavor, and organoleptic properties of instant noodle. The laboratory test results of calcium levels show that 100 grams of catfish meatballs can meet the calcium needs of adolescents aged 10-12 years of 1.6% (P0), 3.05% (P10), 4.25% (P20) and 5.27% (P30). Adolescent calcium intake recommendation is 800 mg (pre-adolescents) to 1,200 mg (adolescents). In the research by Hidayati et al (3), it is stated that calcium intake is related to the incidence of dysenorrhea in adolescent girls where the higher calcium intake, the lower the pain felt by young woman or no pain felt.

Based on observation, the treatment with up to 30% tempeh flour (P30) has the most protein, whereas the treatment without tempeh flour contains the least protein (P0). The protein content influences the increase in protein content in catfish meatballs in tempeh as a substitute for tapioca flour. Thus, if more and more tempeh flour is added to the catfish meatball mixture, the protein content of catfish meatballs will increase. The nutritional content of tempeh is also classified as high, namely 20.8g protein per 100g and calcium content of 155mg per 100g (5). This is in line with the research of (16), which states that the more significant the protein content in the white bread (17). Catfish meatballs are treated in various ways using tempeh

flour according to SNI 7266: 201415, which specifies a minimum protein level of 7% for fish meatballs.

Based on laboratory analysis, it is known that the water content decreases with the addition of tempeh flour to catfish meatballs. The highest water content in catfish meatballs was in treatment P0, and the lowest water content was in treatment P30. Food ingredients that can bind water cannot be separated from the role of protein. Proteins that can bind water are due to groups that have hydrophilic properties and are charged. This shows that tempeh flour can bind or reduce the water content in food. Catfish meatballs with the addition of tempeh flour in various treatments are under SNI 7266: 2014, namely the maximum water content requirement for fish meatballs is 65%.

Taste is the response of the taste buds to nerve stimuli, such as sweet, bitter, and sour. From the Hedonic Scale Test data, it is known that the catfish meatballs that taste the most favored by the panelists are meatballs with the addition of 10% of tempeh flour (P10) with the highest value of 3.56, which means that they have the usual assessment criteria to like the product. This is supported by (18), who said that the addition of tempeh flour at level 1 (the lowest) is the most preferred product (8). The addition of tempeh flour, which is high in protein and fat, can affect the taste. However, the proportions of 20% and 30% of tempeh flour received preliminary ratings by the panelists because the distinctive taste of tempeh flour can mask the distinctive taste of catfish.

The color of food ingredients is frequently used to determine their quality; the color should not depart from the color, which should provide the impression of a separate assessment by the panelists (19). The Hedonic Scale Test revealed that the catfish meatballs with the highest color acceptance were those with a 10% tempeh flour (P10) addition of 3.6. (preferred). Catfish meatballs without tempeh flour (P0) got the lowest color acceptability rating of 3.4. (regular-like). However, with the statistical test of the panelists' assessment of color, there was no difference between the four treatments even though it tended to be disliked along with the addition of substitute materials. This is consistent with (20) confirming that the more Moringa leaf flour added, the lower the preference for the color of the catfish dragon leg (21). Another study by (22) stated that the level of consumer acceptance decreased along with milkfish flour, which was because the more the percentage of milkfish flour was added, the more protein content increased, and the resulting biscuits became darker less attractive (20).

A distinctive and attractive aroma can make food preferred by consumers so that the processing of a food ingredient needs consideration (22). According to the Hedonic Scale Test, the catfish meatballs with 10% tempeh flour (P10) had the highest aroma acceptance, at 3.76 (ordinary - like), while the catfish meatballs with 20% tempeh flour (P20) had the lowest, at 3.36 (ordinary- like), and the addition of tempeh flour can help mask the catfish's fishy aroma. However, there is no significant difference in the assessment. In addition, too high or low temperature will affect the measurement of aroma and flavor (23). The finished dough is then printed roundly and put in water with a temperature of 55-600 C for about 10 minutes. After forming a layer of skin, the meatballs are transferred to water at 80-900 C until the inner meatball temperature reaches  $\pm$  700 C (17). This is supported by (24) that the most preferred aroma of biscuits on additional food program was the control group (without the addition of tofu dregs flour).

The texture of the meatballs that was assessed was chewiness; tapioca flour contained amylose and amylopectin, which made the texture solid and chewy (24). Catfish meatballs with the highest value on texture acceptability were catfish meatballs with 10% tempeh flour (P10) which had a value of 3.48 (regular - like). This is in line with Santi's research that the most preferred edamame nuggets were those with the slightest addition of peanut flour, namely ten grams (25). According to the Indonesian National Standard, the texture of the fish balls is chewy and not mushy either. The addition of tempeh flour has a texture that is almost the same so that it is not significantly different.

The limitation of this study is that this study uses an organoleptic test instrument with 5 criteria, so panelists tend to choose neutral answers.

For future study, iron levels in catfish meatballs with tempeh flour must be determined as iron levels also affect dysmenorrhea in teenage panelists. Thus, the research findings in the form of catfish meatball products can be implemented directly to the target.

To overcome the fishy smell of catfish, for the following product, people can use additional ingredients in the form of ginger so that it will increase the acceptance of catfish meatball products.

### CONCLUSION

The addition of tempeh flour to catfish meatballs as much as 10%, 20%, and 30% can meet 3-5 per cent of the nutritional adequacy rate of adolescents aged 9-12 years on calcium levels. The addition of 10%, 20%, and 30% of tempeh flour fulfils the requirements for fish balls according to SNI for protein content of at least 7%. Catfish meatballs with 10%, 20%, or 30% tempeh flour meet SNI criteria for fish balls with a maximum water content of 65%. In terms of taste, it is not too different in the three formulas, so further research is needed to improve the taste so that the acceptability of catfish meatballs with the addition of 30% will increase. In fact, the most important thing in all products with increased

nutritional value for consumption is taste. Women who suffer from dysmenorrhea might ingest 10-15 grams of catfish meatballs P30 per day to achieve their calcium and protein requirements. This figure is based on the assumption that 100 grams of fish balls contain 63.26 mg of calcium.

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