World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Characteristics of beef meatballs with the addition of moringa leaf flour reviewed in terms of protein, fat, water, total microbia and acidity

Sukisman Abdul Halid [1,*], Minarny Gobel [1], Sugiarto [1] and Abdul Rahim [2]

[1] *Lecturer of the Animal Husbandry Study Program, Faculty of Animal Husbandry and Fisheries, Tadulako University. Jalan Soekarno-Hatta Km.9 Palu 94118, Central Sulawesi, Indonesia.*
[2] *Lecturer of the Agrotechnology Study Program, Faculty of Agriculture, Tadulako University. Jalan Soekarno-Hatta Km.9 Palu 94118, Central Sulawesi, Indonesia.*

World Journal of Advanced Research and Reviews, 2025, 25(02), 1414-1423

Publication history: Received on 05 January 2025; revised on 11 February 2025; accepted on 14 February 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.25.2.0502

Abstract

This study aims to evaluate the physicochemical characteristics and total microbes in beef meatballs with the addition of moringa leaf flour in various ratios. The method used was a Completely Randomized Design with five treatments and three replications, resulting in a total of 15 samples. The parameters measured included protein content, fat content, water content, total microbes (Total Plate Count/TPC), and pH of the meatballs. The results showed that the protein content ranged from 12.30–15.85%, fat content 0.05–0.14%, water content 73.22–79.31%, total microbes 1.63–2.60 log CFU/g, and pH 7.17–7.57. The addition of moringa leaf flour contributed to an increase in protein content and a decrease in fat content and total microbes in meatballs

Keywords: Meatballs; Moringa leaf flour; Protein; Fat; PH; Microbes

1. Introduction

Processed meat food ingredients generally have high nutritional value in terms of protein, amino acid, fat, and mineral content. The increasingly modern era and increasingly busy society, especially in big cities, have resulted in less time to prepare food with a long and time-consuming process. On the other hand, society is increasingly open to healthy and highly nutritious food, but can be served quickly, for example meatballs. Meatballs are a very popular type of food in Indonesia. Meatballs are made from a mixture of no less than 50% meat and starch or cereal flour, with or without permitted food additives [1].

Generally, meatballs are round in shape. However, currently the shape of meatballs is increasingly varied, as is the taste. Meatballs are usually served with noodles or rice vermicelli, vegetables, and broth. Meatballs were introduced to Indonesia by immigrants from China. Meatballs are sometimes made by adding hazardous chemicals such as borax and formalin to make them last longer. This method is not allowed because these preservatives are not for food, so they are dangerous to health. One effort to increase the nutritional value of meatballs, especially the mineral content such as calcium and iron, is by substituting moringa leaves [2]. It is also reported that moringa leaves, in addition to having great potential in processing meatballs, also have the potential as a meatball preservative due to the antioxidant content of moringa leaves [3, 4].

In Indonesia, the use of moringa is still not widely known, generally only known as one of the vegetable menus. In addition to being consumed directly in fresh form, moringa can also be processed into flour or powder that can be used in various food products, such as pudding, cake, nuggets, biscuits, crackers and other processed foods. According to [5],

* Corresponding author: Sukisman Abdul Halid

moringa leaf flour can be added to every type of food as a nutritional supplement. Moringa leaves have high nutrition because according to [5], moringa leaves contain vitamin A which is equivalent to 4 times the vitamin A found in carrots, equivalent to 4 times the calcium found in milk, equivalent to 3 times the potassium in bananas, equivalent to 2 times the protein found in yogurt, and equivalent to 3 times the iron in spinach [6]. In terms of its nutritional content, moringa leaves have great potential to be developed into a mixture of ingredients for making dry noodles. This can increase the nutritional value of noodles and increase the added value of the moringa leaves themselves. Based on the problems above, the author is interested in studying the making of beef meatballs with the addition of moringa leaf flour and evaluating the resulting beef meatballs in terms of protein content, fat content, water content, total microbes, and pH of the meatballs. This study aims to obtain the ratio of tapioca starch to moringa leaf flour in making beef meatballs produced in terms of protein content, fat content, water content, total microbes, and pH of the meatballs.

2. Material and methods

This study used beef obtained from the local market of Palu, moringa leaves obtained from the valley of Palu and tapioca obtained from the supermarket of Palu city. Additional materials used include salt, pepper, onion powder, ice cubes and sodium tripolyphosphate (STPP). Materials for analysis include petroleum ether solvent, Na2CO3 alkali, folin ciopcalteau, distilled water and pure phenol.

2.1. Research Design

This study used a Completely Randomized Design (CRD) method with one factor, namely the ratio of tapioca flour to moringa leaf flour including B0 (15:0), B1 (14:1), B2 (13:2), B4 (12:3), and B5 (11:4) %, consisting of 5 treatments repeated 3 times. Statistical data analysis used Statistical Product and Service Solution software version 13 with the One Way Anova method and Univariate Analysis of Variance with a significant level of 5% in the comparison of means using the Duncan method [7]. The formulation of making meatballs with various additions of moringa leaf flour can be seen in Table 1.

Table 1 The formulation of making meatballs with various additions of moringa leaf flour

Materials	Ratio of tapioca to moringa leaf flour (%)				
	B0	B1	B2	B3	B4
Tapioca (g)	15	14	13	12	11
Moringa leaf flour (g)	0	1	2	3	4
Fresh beef (g)	500	500	500	500	500
Salt (tp)	1	1	1	1	1
Pepper (tp)	0.5	0.5	0.5	0.5	0.5
Garlic powder (sm)	1	1	1	1	1
Ice cubes (g)	40	40	40	40	40
STTP (g)	1.5	1.5	1.5	1.5	1.5

Note: tp (teaspoon), B (beef meatball)

2.2. Preparation for making moringa leaf flour

Moringa leaves that have been obtained from the tree are then washed with running water until clean. Moringa leaves that have been cleaned are dried using an oven blower at a temperature of 50-55 °C for 4-5 hours. The dried moringa leaves are crushed using a blender and then sieved using an 80 mesh sieve to obtain moringa leaf flour. Moringa leaf flour is then stored in a desiccator ready to be used as an additional ingredient in making meatballs.

2.3. Preparation for making meatballs

The method of making meatballs uses a method developed by [8]. Fresh beef that has been separated from fat and connective tissue is cut into small pieces, then put into a food processor by adding ice cubes, STPP and salt then ground for 5 minutes. The next process, filler ingredients (tapioca flour, moringa leaf flour, pepper, and garlic powder) and ice cubes are added then kneaded until smooth. The dough is stored in a refrigerator at a temperature of 10 °C for 10 minutes. Water is heated to a temperature of 100 °C then the water temperature is lowered to 70-80 °C. The dough is formed into balls then heated at a temperature of 70°C for 10 minutes. The balls are boiled at a temperature of 90°C until the meatballs float and are cooked for 15 minutes.

2.4. Protein content analysis

Total nitrogen content was determined by the semi-micro Kjeldahl method. Approximately 1.0 g of samples with a 10 g mixture of potassium sulfate and copper sulfate (100:7) as the catalyst was digested in 25 ml concentrated sulfuric acid at 420 °C. After digestion, cooled samples were diluted with 75 ml of distilled water, distilled into 1% boric acid and titrated against 0.5 M hydrochloric acid. The bromocresol green and methyl red indicators were used, and changed to a final color of light pink at the end point of the titration. The percentage of total nitrogen and of crude protein was calculated using the formula:

$$\% \text{ Total Nitrogen} = \frac{(14.01 \text{ x M x } 100) \text{ x (mL titrant} - \text{mL blank) x } 100}{\text{mg sample}}$$

where 14.01 = the atomic weight of the nitrogen and M = the molarity of the acid (mol/L).

% Crude Protein = Total N x 6.25

Since, on the average, protein contains about 16% nitrogen, one can either divide the percentage of nitrogen by 0.16 or multiply it by a factor of 6.25 to obtain the crude protein content.

2.5. Fat content determination

Fat content analysis was carried out using the AOAC method [9]. The sample was ground and weighed with a weight of 2 g. then mixed with 8 g of sand that had been heated and put into a soxhlet extraction tube in Timble. The coolant was flowed through the condenser. Install the extraction tube on the soxhlet distillation apparatus with sufficient petroleum ether for 4 hours. After the residue in the extraction tube was stirred, the extraction was continued for another 2 hours with the same solvent. Petroleum ether containing fat and oil extracts was transferred into a clean weighing bottle with a known weight and then evaporated with a water heater until thick. Continue drying in a 100 °C oven until constant weight. The weight of the residue in the weighing bottle is stated as the weight of fat and oil.

2.6. Moisture content determination

Water content analysis was carried out using the AOAC method [9]. Clean porcelain crucibles were oven dried at 105 °C for 2 h, cooled in a dessicator and weighed (W0). Approximately 2.0 g of samples was weighed into the crucibles and the whole crucible with the sample weighed again (W1). The crucibles with the samples were oven dried at 105 °C for 8 h, and cooled in a dessicator before being reweighed (W2). The moisture content was calculated with the following formula and expressed as the percentage per gram sample.

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1 - W_0} \text{ x } 100$$

2.7. Total microbial determination

Total microbial testing was carried out according to the method developed by [10]. Sterilization of tools and materials is done by wet method, namely by inserting tools and materials into autoclave at 121°C, pressure 2 atmospheres for 15 minutes. Preparation and planting of samples are done aseptically. Preparation of samples is done by dilution method, including: Dilution 10^{-1} is made by transferring 5 g sample into test tube I containing 45 ml of 0.1% peptone, shaken until homogeneous. Dilution 10^{-2} by transferring 1 mL of solution from dilution 10^{-1} into test tube II containing 45 ml of 0.1% peptone until dilution 10^{-6}. Planting is done by pipetting 1 mL of sample solution from each dilution into each petri dish. Next, agar media is poured, then rotated to spread the sample in the agar media. After freezing, it is inoculated at 37 °C for 48 hours with the dish inverted. Colonies that grow after incubation, using Stuart Scientific Colony Counter. The number of colonies included in the calculation is between 25 to 300 colonies. The number of microbes per mL is obtained by multiplying the number of colonies per plate by the dilution factor. Dilutions that produce less than 25 colonies, only the lowest dilution is counted. The number of colonies is more than 300 colonies, then the highest dilution is counted. Two levels of dilution produce colonies with a number between 25 to 300 colonies, then the average of the two dilution values is counted.

2.8. pH analysis

pH was determined by using pH meter of AOAC Method [9], where 1 g of bioplastic was diluted into distilled water until the volume up to 10 mL. The pH of the samples was determined when the pH monitor showed a constant value.

3. Results and discussion

3.1. Beef meatball protein content

The protein content of meatballs made using a mixture of beef and Moringa oleifera leaf flour shows that variations in the concentration of Moringa leaf flour have different protein levels between treatments. The average value of the protein content of the beef meatballs produced is presented in Table 2. The results of the analysis of protein content variance showed significant differences ($p \leq 0.05$) in the five meatball treatments. Variations in the concentration of moringa leaf flour in making beef meatballs produced different protein levels. The highest protein content was obtained in treatment B2, which was 15.85 + 0.67%, and the lowest was obtained in treatments B1, B3, and B4, respectively, at 14.35 + 0.39%, 13.03 + 0.33%, and 13.17 + 1.97%. Meanwhile, treatment B0 (without moringa leaf flour) only had a lower protein content, which was 12.30 + 0.67%. The results of this study indicate that the use of moringa leaf flour in making meatballs produces different protein levels in meatballs.

Table 2 Average protein content of beef meatballs processed with a mixture of moringa leaf flour

Tapioca ratio to Moringa leaf flour (%)	**Protein Content (%)**
B0 (15:0)	12.30±0.67b
B1 (14:1)	14.35±0.39c
B2 (13:2)	15.85±0.67d
B3 (12:3)	13.03±0.33b
B4 (11:4)	13.17±1.97a

Different letter notations after numbers in the same column indicate significant differences ($p \leq 0.05$).

The difference in protein content of meatballs produced by adding different concentrations of moringa leaves is due to the raw material of moringa leaf flour used having a high protein content, thus affecting the protein content of the meatballs produced. Moringa leaves contain 60.34% protein, so the more concentration of moringa leaf protein added, the protein content of beef meatballs substituted with moringa leaf flour also increases [11, 12].

This study also shows that the higher the concentration of moringa leaf flour in making meatballs is followed by a decrease in protein content. The decrease in protein content of beef meatballs according to [13]. is due to the addition of salt ranging from 7.12% to 9.80%. The higher the addition of NaCl, the lower the protein content of the beef meatballs produced. NaCl weakens the interaction between protein groups with different charges. Chloride ions in salt that are negatively charged will bind to the positive protein group and cause the total protein charge to become negative so that there is a repulsive force between the filaments. This repulsive force causes the space between the filaments to open, thus providing more space to bind more water.

According to [14], the protein content of meatballs according to SNI 01-3818-1995 is a minimum of 9%. Based on the SNI meatball standard, it shows that beef meatballs processed with moringa leaf flour still have a protein content according to SNI meatballs. The difference in protein content of meatballs is thought to be due to the use of tapioca flour as a thickener. The protein content of meatballs decreased along with the addition of tapioca flour [15]. According to [16], the higher the amount of tapioca flour added, the lower the protein content of meatballs or the lower the proportion of meat.

3.2. Fat content of beef meatballs

The fat content of meatballs made using a mixture of beef and Moringa oleifera leaf flour showed that the higher the concentration of Moringa leaf flour, the resulting meatballs had a lower fat content between treatments. The average value of the fat content of the beef meatballs produced is presented in Table 3.

Table 3 Average value of fat content of beef meatballs prepared with a mixture of Moringa leaf flour

Tapioca ratio to Moringa leaf flour (%)	Fat content (%)
B0 (15:0)	0.14±0.02b
B1 (14:1)	0.14±0.02b
B2 (13:2)	0.07±0.01a
B3 (12:3)	0.07±0.01a
B4 (11:4)	0.05±0.01a

Different letter notations after numbers in the same column indicate significant differences (p≤0.05).

The results showed significant differences (p≤0.05) in five meatball treatments. The higher the concentration of moringa leaf flour, the lower the fat content. The highest fat content was obtained in treatments B0 and B1, which were 0.14 + 0.02%, respectively, and the lowest was obtained in treatments B2 and B3, which were 0.07 + 0.01% and 0.07 + 0.01%, respectively. The fat content of meatballs is always related to the water content. If the water content is high, it will be followed by a lower fat content [17]. Added 30% tapioca flour50% did not affect the fat content of meatballs, this is likely because the fat content of tapioca flour and moringa leaf flour is very low so that it affects the fat content of the meatballs produced. The next lowest fat content of meatballs is the B4 treatment, which is 0.05 + 0.01% [18].

This study showed that the higher/more moringa leaf flour substituted in making beef meatballs, the fat content of the resulting meatballs decreased, this can be caused by the fat content of moringa leaf flour and the fat content of tapioca flour interacting with each other in binding fat in the meatball processing process. Tapioca flour and moringa leaf flour together are thought to bind fat so that meatball fat decreases. According to [19], fiber can bind fat and protein, so that fat in meatballs decreases. However, the decrease in fat content in meatballs with the addition of moringa leaf flour in each treatment was statistically significant.

In addition, the effect of boiling on water content can cause meat shrinkage so that a lot of water comes out of the meat, in addition, a lot of water also evaporates during boiling. According to [20] reported that the heating process of additional additional materials in the processing of food products causes fat to melt and its viscosity to decrease, making it easier for fat to come out. However, the meatball products produced, based on the SNI meatball standard according to SNI 01-3818-1995 that the criteria for the fat content of meatballs produced is a maximum of 2% b/b. The fat content of the beef meatballs produced when compared to the SNI meatballs is still in the range of SNI meatballs.

3.3. Water content of beef meatballs

The water content of meatballs made with a mixture of beef and Moringa oleifera leaf flour varies between treatments. The average value of the water content of the beef meatballs produced is presented in Table 4.

Table 4 Average water content of beef meatballs processed with a mixture of moringa leaf flour

Tapioca ratio to Moringa leaf flour (%)	Water content (%)
B0 (15:0)	74.77±1.83a
B1 (14:1)	76.67±1.91ab
B2 (13:2)	73.22±0.77a
B3 (12:3)	75.84±0.65a
B4 (11:4)	79.31±2.85b

Different letter notations after numbers in the same column indicate significant differences (p≤0.05).

The results showed that the treatment of beef meatballs with the addition of moringa leaf flour had a significant effect (p≤0.05) on the water content of the meatballs. This shows that the higher the addition of moringa leaf flour to beef meatballs affects the water content of the meatballs produced. The highest water content was obtained in treatment B4, which was 79.31 + 2.85%, and the lowest was obtained in treatments B1, B3, and B2, respectively, which were 76.67 + 1.91%, 75.84 + 0.65%, and 73.22 + 0.77%. While in treatment B0 (without moringa leaf flour) the water content was

74.77 + 1.83%. Based on SNI 01-3818-1995, the water content of meatballs that must be met is a maximum of 70% w/w.

The highes water content of the meatballs is thought to be influenced by the water content of the raw materials for making meatballs, so the addition of beef, moringa leaf flour, and tapioca flour with different percentages affects the water content of the meatballs. It was reported that beef has a water content of 74.16 [21], while the water content of moringa leaves is 8.35% [22]. The highest protein content of moringa leaves was obtained in old moringa leaves, which was 11.35% and the lowest protein content was obtained in young moringa leaves, which was 1.31% [23]. According to [24], moringa leaves contain various amino acids that are hydrophobic and hydrophilic. In the process of making moringa leaf protein concentration, hydrogen bonds are broken which causes changes in hydrophobic properties due to the protein denaturation process, so that there is a change in the level of hydrophobicity which results in more water being bound. According to [1], the water content of meatballs is greatly influenced by chemical compounds, temperature, consistency, and interactions with food components such as protein, fat, vitamins, free fatty acids and other components. Furthermore, the decrease in water content of meatballs is thought to be due to tapioca flour and moringa leaf flour functioning as binding agents that can increase water binding capacity.

The decrease in water content is due to the interaction mechanism of starch and protein, so that water cannot be bound perfectly because the hydrogen bonds that should bind water have been used for the interaction of starch and protein [25]. Thus, the higher the percentage of flour used, the greater the mass of flour in the meatballs and the water content of the meatballs will decrease. The decrease in water content is also caused by the starch contained in the flour increasing the total weight and absorbing water, while the water content in the meat remains the same. As a result, the water content decreases [26].

3.4. Total microbes of beef meatballs

Total microbes (*Total Plate Count/*TPC) of meatballs made with a mixture of beef and moringa leaf flour (Moringa oleifera) varied between treatments. The average value of the total microbes of the beef meatballs produced is presented in Table 5. The results showed that the treatment of beef meatballs with the addition of moringa leaf flour had a significant effect ($p \leq 0.05$) on the amount of TPC. The results showed that the concentration of moringa leaf flour in beef meatballs affected the TPC of the meatballs produced. The highest TPC was obtained in treatments B0 (without moringa leaf flour), B2, and B4. which were respectively 2.30 + 0.20 log cfu/g, 2.23 + 0.06 log cfu/g, and 2.60 + 0.26 log cfu/g. While the lowest TPC was obtained in treatments B1 and B3, respectively 1.63 + 0.06 log cfu/g and 1.63 + 0.15 log cfu/g.

Table 5 Average total microbial value of beef meatballs processed with a mixture of moringa leaf flour

Tapioca ratio to Moringa leaf flour (%)	TPC (cfu/g)
B0 (15:0)	2.30±0.20bc
B1 (14:1)	1.63±0.06a
B2 (13:2)	2.23±0.06b
B3 (12:3)	1.63±0.15a
B4 (11:4)	2.60±0.26c

Different letter notations after numbers in the same column indicate significant differences ($p \leq 0.05$).

All food products have regulations on the maximum TPC limit according to SNI [27]. According to the Indonesian National Standard (SNI) 01-3818-1995, the quality requirements for meatballs are a maximum of 1 x 105 log cfu/g. The number of microbial populations when mucus is formed is 3.0 x 106 to 3.0 x 108 colonies/gram sample and the number of microbial populations when an unpleasant odor is detected is 1.2 x 106 to 108 colonies/gram. visually, mucus has formed [28]. According to [29], if mucus has formed on meatballs, it indicates that the meatballs have experienced a decline in quality due to bacterial activity and are not suitable for consumption.

The high TPC content is suspected of implementing a sanitation program starting from handling raw materials to the processing process which requires handling by applying basic hygienic principles based on the Hasards analysis critical control point (HACCP) system [30]. The total microbes need to be known to ensure that a food ingredient is fit for consumption [31]. It is reported that meatballs are round or other shaped food products, obtained from a mixture of livestock meat (meat content not less than 50%) and starch or cereals with or without the addition of permitted food.

In addition to protein, carbohydrates, and vitamins, meatballs also contain high water content so that meatballs are placed as food in the Intermediate Moisture Food (IMF) group or semi-wet food. Food like this has a higher aw, namely 0.90 and food like this will encourage bacterial growth. Therefore, meatballs are a good medium for bacterial transfer [32].

The increase in microbial contamination of meatballs comes from microbial contamination during the meatball making process. The source of contamination is likely from tools or materials added (tapioca, spices or other ingredients) during the processing process which can also come from less hygienic processing methods [15]. Contamination can occur if the finished food produced is in direct contact with the surface of the table or food processing equipment during the preparation process that has previously been contaminated by microbes. The process of microbial contamination in meatballs can occur through the meatball making place and the level of contamination comes from each source and depends on the sanitation method used. A very significant source of contamination is from surfaces that are in direct contact with the meatballs [33].

3.5. pH of beef meatballs

The pH of meatballs processed with a mixture of beef and Moringa oleifera leaf flour showed that variations in the concentration of Moringa leaf flour had different protein levels between treatments. The average pH value of the beef meatballs produced is presented in Table 6. The results showed a significant effect ($p \leq 0.05$) in the five meatball treatments on the pH of the meatballs. The concentration of Moringa leaf flour and tapioca flour in making beef meatballs obtained different meatball pHs. The highest meatball pH was obtained in treatment B2, which was 8.21 + 0.15, and the lowest was obtained in treatment B1, which was 7.17 + 0.13. While in treatment B0 (without Moringa leaf flour) it had a pH of 7.57 + 0.41. The results of this study indicate that the use of Moringa leaf flour in making meatballs produces different meatball pH.

Table 6 Average pH value of beef meatballs prepared with a mixture of Moringa leaf flour

Tapioca ratio to Moringa leaf flour (%)	pH
B0 (15:0)	7.57±0.41b
B1 (14:1)	7.17±0.13c
B2 (13:2)	8.21±0.15d
B3 (12:3)	7.45±0.46b
B4 (11:4)	7.39±0.63a

Different letter notations after numbers in the same column indicate significant differences ($p \leq 0.05$).

Measurement of pH value is done to determine the acidic, neutral or basic nature of a food product produced. According to [31] reported that pH value is an important indicator that needs to be considered, because changes in pH affect the quality of the meatballs produced. The pH value of meatballs according to the Indonesian National Standardization [34] is between 6 and 7, this means that the pH value of beef meatballs processed with a mixture of moringa leaf flour still meets the pH limits according to the Indonesian National Standardization.

The results of the study showed that the pH value of meatballs varied according to the variation in the concentration of moringa leaf flour and tapioca flour added. This is influenced by the basic ingredients used, namely meat and flour. According to that the pH value of the meat dough is influenced by the ingredients used, especially the pH of the meat used and the pH value of the meatball dough is also related to the water binding capacity of the meatballs.

According to [16], the pH value of the basic ingredients of meatballs will cause changes in the pH value of the meatballs. Changes in the pH of meatballs according to [29] are due to changes in the hydrogen balance in meatballs as an influence of the pH value of the basic ingredients used in making meatballs. The decrease in the pH of meatballs, besides being influenced by the mixing of ingredients to create a new hydrogen balance point in meatballs, is also influenced by the presence of fast microbial metabolic activity, so that the pH of meatballs decreases [35].

According to [36] stated that changes in meatball pH are also influenced by the quality of technology and the quality of fresh meat affects the pH of the resulting product. Treatment B2 has a higher pH than other treatments. [37] stated that meatballs that have not undergone a storage process have a higher average pH value compared to meatballs that have undergone a storage process for 2 or 3 days. [38] stated that high temperatures increase the rate of pH decline, while low temperatures inhibit the rate of pH decline.

4. Conclusion

The results showed that meatballs with the addition of moringa leaf flour had better physicochemical characteristics compared to meatballs without the addition of moringa leaf flour. Protein content increased with the addition of moringa leaf flour, while fat content decreased. In addition, the total microbes in meatballs with moringa leaf flour tended to be lower, indicating that moringa leaves can act as an additional ingredient with antimicrobial potential. Overall, meatballs with the formulation of tapioca and moringa leaf flour ratio produced better meatball quality than the control.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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