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Total Plate Count (TPC) and Organoleptic Test on Beef Meatballs with Moringa (*Moringa oleifera*) Leaf Flour Addition

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ABSTRACT: Meat refers to parts of an animal's body, such as liver, kidneys, brain, and other muscle tissues that are edible, and meat has a complete nutritional content. Meatballs are a type of food popular in Indonesia, but are prone to spoilage due to the activity of microorganisms. The production of beef meatballs with the addition of moringa leaf powder (Moringa oleifera) can inhibit microbial growth and affect the quality of beef meatballs. This study aims to evaluate the effect of *Moringa oleifera* (Moringa leaf flour, MLF) addition on total microbes and organoleptic of beef meatballs, including color, texture, taste, and preference for color, aroma/smell, texture, and flavor. The research method used a completely randomized design (CRD) with four treatments: meatballs without the addition of MLF (T0) and meatballs with the addition of MLF 1% (T1), 2% (T2), and 3% (T3). The parameters observed included measurements, total microbes, and organoleptic characteristics, which included hedonic and sensory quality tests of beef meatballs. The results showed that the addition of moringa flour had a significant effect ($P < 0.05$) on the hedonic and organoleptic quality tests, but had no significant effect ($P > 0.05$) on total microbes. The study results show that adding MLF at different levels does not effectively suppress the microbial growth of beef meatballs. Adding MLF with different levels in beef meatballs does not effectively suppress microbial growth. The addition of MLF in the hedonic and organoleptic quality tests significantly affected colour, texture, smell, and taste, and was still acceptable to the panellists.

Keywords: Meatballs, moringa leaf flour, total microbes, organoleptic test.

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INTRODUCTION

Meat is part of the animal body, including the liver, kidney, brain, and other muscle tissue that can be eaten. Meat is reported to have a relatively complete nutritional content, consisting of 75% water, 19% protein, 3.5% soluble non-protein, and 2.5% essential amino acids, vitamin B12, niacin, vitamin B6, iron, zinc, and phosphorus. (Halid et al., 2023) The high content of nutrients in this product makes meat a favorite among all ages. One of the most popular foods in Indonesia is the meatball. Generally, meatballs in the market are made from beef and chicken. (Rasak et al., 2023) Beef meatballs are made from ground beef, to which spices and tapioca flour are added to form a dough. This dough is then formed into small balls and boiled until cooked, as indicated by the meatballs floating to the surface. (Agustina et al., 2013).

Beef meatballs are susceptible to spoilage by microorganisms when stored at room temperature. Therefore, the manufacturing process can control the inhibition of spoilage in beef meatballs. One of the natural ingredients that can inhibit the growth of microorganisms is Moringa leaves. According to Aminah et al.

(2015), as an antimicrobial, Moringa leaves have a pharmacological effect, containing ascorbic acid, flavonoids, phenolics, and carotenoids.

Local plants such as Moringa leaves (*Moringa oleifera*) contain high nutritional content and nutrients that can significantly benefit health. Moringa leaf production is to produce moringa leaf flour. Moringa leaf flour is produced by drying moringa leaves and then crushing and blending them until smooth. Research by Jannah et al. (2020) Said that moringa plants are easy to grow and do not die easily, even though they are planted on nutrient-poor land. Moreover, Moringa is rich in nutrients because it contains essential phytochemical substances in its leaves, pods, and seeds. The plant contains vitamin C, about 5.81 - 6.60 mg/g, phenolics about 36.02 - 45.81 mg/g, flavonoids about 15 - 27 mg/g, and beta-carotene about 42.08 µg/g (Rasak et al., 2023).

In Indonesia, many studies have been conducted on natural materials, mainly because plants produce many active compounds through secondary metabolism that provide health benefits. One of them is the Moringa plant, which has been shown to have anti-cancer, antibacterial, and antihypertensive properties and the ability to inhibit bacterial and fungal activity. The chemical content in moringa leaves includes high concentrations of vitamins A and C. According to Beti et al. (2020) Reported that the addition of Moringa leaf extract at concentrations of 0%, 5%, 10%, and 15% significantly influenced the Total Plate Count (TPC) values, indicating a notable impact on the microbiological quality of the product. The results of research by Djawa et al. (2021) showed that the provision of moringa leaf flour in the organoleptic meatballs of abandoned laying hens significantly affects the categories of color, smell, texture, and taste. Therefore, a study was conducted to investigate the addition of moringa flour and its effect on the total microbes and organoleptic properties of beef meatballs.

This study aims to determine the effect of adding *Moringa oleifera* flour on the quality of beef meatballs. The addition of Moringa oleifera flour is expected to inhibit microbial growth and enhance the organoleptic properties of beef meatballs.

MATERIALS AND METHODS

Time and Place of Research

This research was conducted from February 22, 2024, to June 30, 2024, at the Laboratory of the Department of Animal Husbandry, Faculty of Agriculture, Bengkulu University.

Tools and Materials

The equipment used in this study includes a food processor, a pot for boiling, a stove, digital scales, a plastic basin, a kitchen knife, a tray, a spoon, a plastic container for samples, a pH meter, a measuring cup, a ruler, a pencil, a tissue, a sterile pipette, a petri dish, an incubator, and a colony counter. This study used 6.4 kg of beef, tapioca flour, ice cubes, salt, pepper, garlic, Moringa leaf flour, distilled water, NaCl, and PCA (Plate Count Agar).

Preparation of Moringa Leaf Flour (MLF)

The Moringa leaves **are** first subjected to sun-drying for 72 hours, followed by **oven-drying at 60°C for 4 to 5 hours. Subsequently, the dried leaves are** ground into flour using a blender and sieved through a 35-mesh screen to obtain a fine and uniform particle size.

Meatball Formula and Treatments

The formulation and composition of meatballs can be seen in Table 1 below using the modified method of Murti et al. (2013).

Table 1. Meatball formula and treatment of Moringa Flour addition.

Material	Treatment			
	T0	T1	T2	T3
Meat (g)	400	400	400	400
Tapioca flour (g)	200	200	200	200
Ice cubes (g)	104	104	104	104
Table salt (g)	12	12	12	12
Garlic powder (g)	4	4	4	4
Pepper powder (g)	4	4	4	4
Moringa Leaf Flour (g)	-	4 (1%)	8 (2%)	12 (3%)

Meatball Making

Beef is cut into small pieces, ground, and crushed. Salt and 1/3 of ice are added. The dough is then mixed with 1/3 of ice, powdered moringa leaves according to the treatment, garlic, and pepper, and crushed for 2 minutes. The dough is mixed with 1/3 of ice and tapioca, crushed until smooth (3 minutes). The dough is allowed to rest for 10 minutes, molded into a round shape, and boiled in boiling water for approximately 10 minutes. All stages of making meatballs were carried out in phases and adjusted to the treatment and replication in the study.

Research Design

The study used a completely randomized design (CRD) consisting of 4 treatments, each repeated 4 times. The treatments were differentiated based on the level of addition of moringa flour into the meatball dough, namely:

T0: Meatballs without MLF addition.

T1: Meatballs with the addition of MLF 1% of meat ingredients.

T2: Meatballs with the addition of MLF 2% of meat ingredients.

T3: Meatballs with the addition of MLF 3% of the meat ingredients.

Variables observed

Total Microbes

Total Plate Count (TPC) is determined as follows: a sample with a dilution of 10-1 is *prepared by adding a sample of 5 grams and putting it* into a NaCl solution of 45 ml, then homogenized using a Stomacher for 2 minutes. The subsequent dilution of 10-2 is *achieved by adding 1 ml of the sample to a 9 ml NaCl solution*. Then, *homogenize* it using a stomacher for 2 minutes. Likewise, with dilutions 10-3 and 10-4, they are then put into a *Petri* dish. 12 - 15 ml of PCA, *cooled in a water bath to a temperature of 45 °C,* was added to each cup containing the solution/sample. After solid agar, the dishes were incubated for 48 hours at *35* °C. The number of bacteria was counted using a Colony Counter tool. (Khotimah et al., 2019).

Organoleptic Test

This organoleptic test was used to determine consumer preference for the processed products produced. The test included color, smell/aroma, taste, and smoothness/texture. It involved 25 panelists selected from students in the Department of Animal Husbandry, Faculty of Agriculture, Bengkulu University, who had taken the Basic Animal Product Technology course. The tests were conducted using the line scale method to observe both hedonic and sensory quality.

Hedonic Quality Test: this test includes color, texture, and taste, made with the following line scale:

Color

Very dark — Normal — Very light

1 3 5 7 9

Texture

Very rough — Normal — Very fine

1 3 5 7 9

Taste

Very bad — Normal — Very good

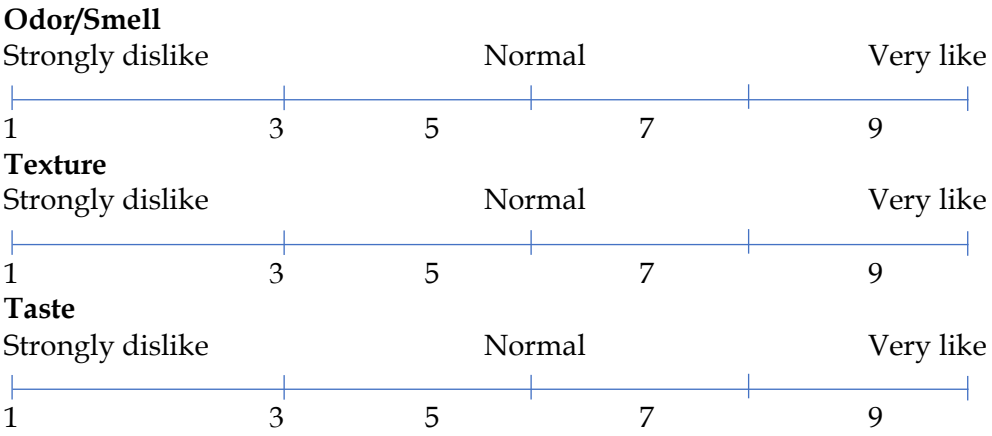
1 3 5 7 9

Hedonic test: this test includes color, aroma, texture, and taste, made with the following line scale:

Color

Strongly dislike — Normal — Very like

1 3 5 7 9



Data Analysis

Organoleptic tests were conducted to evaluate the author's liking for the meatballs produced. The data was then analyzed using Analysis of Variance (ANOVA). If the results show a significant effect of the treatment, the Duncan's Multiple Range Test (DMRT) will be continued. DMRT helps determine substantial differences between different treatments and can identify the treatment that gives the most favorable results, according to the author (Steel and Torrie, 1994).

RESULTS AND DISCUSSION

Total Microbes of Beef Meatballs

The total number of microbes in beef meatballs is a crucial indicator of product quality and safety. The determination of total microbes is performed using the Total Plate Count method (TPC), which is commonly used to measure the total number of microbes in food samples. Data from the TPC test on beef meatballs supplemented with moringa flour are presented in Table 2.

Table 2 shows that the total number of microbes in the meatballs is still below the maximum limit. This indicates that the meatball product is safe to use. This shows that the meatball products meet established standards of microbiological hygiene and safety, whether Moringa flour is used. According to SNI (2014)The maximum value of total microbes in beef meatballs is 1 x 10^5 colonies/gram. The total microbes in this study yielded results between 3.20 and 3.80 log cfu/g, or approximately 10^3 colonies/gram. In this study, the total microbes did not exceed 10^5, so beef meatballs with Moringa flour still meet safety standards and are safe for consumption.

The results of the TPC test on beef meatballs with moringa leaf flour did not have a significant effect ($P>0.05$) in suppressing microbial growth. Supplementation in the form of moringa leaf extract can inhibit microbial growth. The compounds present in Moringa leaf extract may act as antimicrobial agents. These compounds include alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, tannins, triterpenes, and sterol saponins. Polyphenols in Moringa leaf extract can inhibit microbial growth by molecular inhibition, which occurs mainly in the cell membrane of microorganisms. (Agustina et al., 2013). This can cause malformations in microbes and increase the permeability of their membranes. The limit for microbial contamination in processed meat products is usually 10^5 colonies per g. Given the antimicrobial properties of Moringa leaf extract, the addition of this extract to processed meat products can help ensure that the product passes the test against the set microbial contamination limit.

According to Asmaq et al. (2023)The compounds present in Moringa leaf meal have the potential to inhibit microbial growth in lamb nuggets. However, the study's results, which added moringa flour to beef meatballs, were ineffective in suppressing microbial growth in beef meatballs.

Table 2. *Total Plate Count of* beef meatballs

Variables	Treatment			
	T0	T1	T2	T3
Total Microbes (log CFU/g)	3.80±0.82	3.20±1.30	3.65±2.98	3.57±4.09

Description: T0: meatballs without MLF, T1: addition of 1% MLF, T2: addition of 2% MLF, T3: addition of 3% MLF.

Hedonic Quality Organoleptic Properties

The results of the hedonic quality test on beef meatballs with the addition of moringa flour showed a significant effect (P< 0.05) on the color, texture, and taste categories. Furthermore, the Duncan test revealed significant differences among the three treatments with the addition of moringa flour.

Table 3. Mean properties of Organoleptic Hedonic Quality

Variables	Treatment				T
	T0	T1	T2	T3	
Color	5.39±0.42^{a}	4.38±0.69^{b}	4.11±0.24^{b}	4.08±0.45^{b}	0.007
Texture	5.06±0.27^{a}	4.7±0.33^{ab}	4.35±0.41^{bc}	4.04±0.56^{c}	0.014
Taste	5.05±0.14^{a}	4.56±0.45^{b}	4.17±0.16^{bc}	4.01±0.22^{c}	0.012

Description: Different superscripts in the same row or column indicate significant differences (P<0.05). T0: meatballs without MLF, T1: 1% MLF addition, T2: 2% MLF addition, T3: 3% MLF addition.

Hedonic Organoleptic Properties

The results of the liking test for beef meatballs with the addition of moringa flour had a significant effect (P < 0.05) on the color, texture, smell, and taste categories. Furthermore, the Duncan test revealed significant differences among the four treatments with the addition of moringa flour.

Table 4. Mean of Hedonic Organoleptic properties

Variables	Treatment				T
	T0	T1	T2	T3	
Color	5.23±0.30^{a}	4.48±0.30^{b}	4.15±0.23^{bc}	4.01±0.05^{c}	0.000
Texture	5.06±0.30^{a}	4.98±0.28^{ab}	4.54±0.12^{b}	4.05±0.38^{c}	0.008
Smell	5.07±0.29^{a}	4.55±0.07^{b}	4.39 ±0.27^{b}	4.24±0.33^{b}	0.014
Taste	5.01±0.27^{a}	4.49±0.31^{b}	4.25±0.27^{b}	4.13±0.24^{b}	0.026

Description: Different superscripts in the same row or column indicate significant differences (P<0.05). T0: meatballs without MLF, T1: 1% MLF addition, T2: 2% MLF addition, T3: 3% MLF addition.

The results of the study on beef meatballs with different amounts of Moringa flour showed a very significant effect (P <0.05) on colour in the hedonic quality test. Furthermore, Duncan's test also showed a significant colour difference. The colour score is 5.39-4.08, which is decreasing. The addition of Moringa flour to beef meatballs affects the colour of the meatballs. Panelists generally prefer the light colour of meatballs (T0), while darker coloured meatballs are less preferred. The inclusion of moringa flour in beef meatballs is thought to affect the colour of the meatballs, which in turn affects the panelists' liking of the product. According to Indraswari et al. (2022)The test results in the colour category were highest in the P0 treatment. The dark colour of beef meatballs is influenced by moringa leaf flour, which contains chlorophyll. Chlorophyll, or green pigment, is a compound found in plants. (Krisnadi, 2015). According to Djawa et al. (2021) Chlorophyll is a green pigment found in chloroplasts along with carotene and xanthophyll. The highest results were obtained in untreated beef meatballs. This is because panelists prefer light colours, which are considered more visually appealing. Beef meatballs are generally light in colour, so their visual appearance may be more appetising to some panelists. The success of the untreated beef meatballs in the organoleptic test, which achieved the highest results, is also in line

with the quality standards set by the SNI (2014) For the colour category, where the colour is rated as normal and acceptable by the panellists, this confirms that the product not only meets the panelists' preferences but also meets the established quality standards.

The results showed that in the colour category, there was a very significant effect (P<0.05) in the hedonic test. In addition, Duncan's test showed a very substantial impact for colour. The highest hedonic value in the colour category was obtained in the T0 treatment, which was 5.23 without addition, and the lowest was T3, which was 4.01 with a lot of moringa flour at 3%. The colour in the P0 treatment had the highest level of liking compared to T1, T2, and T3, which decreased. The provision of moringa flour may reduce the panelists' interest and liking of these meatballs, as beef meatballs usually have a bright colour. Food colour is an important indicator that influences the desire to buy and consume the food (Indraswari et al., 2022). According to Hadiansyah et al. (2020), colour influences people's appetite. It is an attraction for shoppers, based on the results of his research, which showed that panelists preferred the nuggets without it to those with additional moringa flour. According to SNI (2014), the quality requirements for beef meatballs are standard. The results of this study showed that the panellists preferred meatballs without the addition of moringa flour.

The results of the study with different amounts of moringa leaf flour showed a significant effect (P <0.05) on the texture attribute of the hedonic quality test. Furthermore, Duncan's test was continued to obtain the results of a significant effect on texture. The texture category received the highest score of 5.06 in the T0 treatment compared to the T1, T2, and T3 treatments. The panellists liked the texture of the meatballs without treatment. The reason for the dislike of the beef meatballs in the T1, T2, and T3 treatments is the excessive amount used. According to Ulfa & Ismawati (2016)The addition of moringa leaves affects the chewiness of the texture. The fewer moringa leaves added, the more chewy the meatballs will be. This is because the starch content is low, so water absorption is also low. And also high in fibre, reaching five times more than other vegetables. So the more flour you add, the more chewy the meatballs will be. According to Indraswari et al. (2022) The results on the texture of fish meatballs are soft, chewy, and not brittle due to the amount of fish meat used instead of moringa flour. Moringa leaf flour contains compounds that play an essential role in tissue structure, contributing to the dense texture of the meatballs. However, these compounds can become brittle if used excessively. (Setiaboma et al., 2021).

The results of the hedonic texture study showed a very significant effect (P <0.05) on the texture attribute in the hedonic test. Furthermore, Duncan's test showed a significant impact on texture. The highest value was obtained in the T0 treatment with 5.06, with the addition of 0%, while the lowest value was obtained in the T3 treatment with 4.05, with the addition of 3%. According to Ulfa & Ismawati (2016) The study reported that lower concentrations of Moringa flour tend to produce meatballs with a firmer, chewier texture, which is generally more acceptable to panelists. The results of this study showed that the panelists preferred the T0 treatment to the T1, T2, and T3 treatments.

The results of the odour category showed a very significant effect (P < 0.05) on the hedonic test. Furthermore, Duncan's test revealed a significant difference in odour. The highest hedonic score in the odour/aroma category was 5.07 in the T0 treatment, and the lowest was 4.24 in the T3 treatment with 2% moringa meal. The odour in the T0 treatment had the highest level of liking compared to T1, T2, and T3, which decreased. This is because the more the odour/flavour is provided, the more the distinctive aroma of moringa is created, which is not liked by the panellists. (Setiaboma et al., 2021). According to Ulfa & Ismawati (2016)The aroma of the meatballs comes from the moringa leaves, which contain lipoxidase enzymes. Green vegetables often contain lipoxidase enzymes, and if the cooking process is not perfect, these enzymes can produce an unpleasant aroma, such as a languorous aroma. In the research by Indraswari et al. (2022) The results of the hedonic evaluation showed that the panellists preferred the aroma of the meatballs in the first treatment compared to the second and third treatments. In the first treatment, the aroma of the meatballs was more dominant than the aroma of moringa. In contrast, in the second and third treatments, the

aroma of moringa was more prominent than the aroma of the meatballs, so panellists tended to prefer the aroma of the meatballs to the aroma of moringa leaves.

The results showed that the colour category of the moringa flour meatballs had a very significant effect (P<0.05) on the taste attributes in the hedonic quality test. Furthermore, Duncan's test still showed a significant difference. The flavour score value obtained from T0 was 5.05, and the lowest result obtained from T3 was 4.01. According to Indraswari et al. (2022), chicken meatballs without treatment are preferred over those with moringa flour added at 5 g and 10 g in the two treatments. It is believed that increasing the amount of moringa flour in the beef meatballs results in a bitter taste. The use of additional moringa flour in making beef meatballs may give a bitter taste to the meatballs because it is believed that moringa flour contains phenolic and alkaloid compounds that provide a bitter taste. Therefore, if too much is added to processed products, a more bitter taste will be felt in the product (Cahyaningati & Sulistiyati, 2020). According to Ulfa & Ismawati (2016), giving too much can affect meatballs because of the presence of tannin compounds. Tannins provide the meatballs with an astringent taste because, when consumed, tannins coagulate the proteins that line the oral cavity and tongue. According to Djawa et al. (2021), tannins tend to cause an astringent taste when consumed because they cross-link with glycoproteins in the oral cavity, resulting in a dry, wrinkled, or astringent sensation.

The taste results showed that providing different amounts of Moringa flour had a very significant effect (P <0.05) in the hedonic test. Furthermore, Duncan's test showed a significant difference in taste. The highest score was obtained in the T0 treatment with 5.01 without moringa flour, and the lowest score was obtained in the T3 treatment with 4.13 with the addition of 3% moringa flour. According to Indraswari et al. (2022)The addition of meatballs affects the panelists' liking because moringa leaves contain tannins that cause an astringent taste. If the amount is increased, it has an unpleasant taste, while if it is not used, it has a delicious flavor (Walten et al., 2023). In this study, the taste of the meatballs in the T0 treatment was preferred by the panellists because there was no addition, while the addition of 3% was not desired. Based on SNI (2014)The taste of the meatballs is typical due to the use of basic ingredients and appropriate seasonings (Walten et al., 2023). In this result, the panellists particularly liked the meatballs without the addition of excessive moringa flour.

CONCLUSION

Based on the study's results, adding moringa flour to beef meatballs at concentrations of 1%, 2%, and 3% does not effectively inhibit microbial growth, but it still meets safety standards and can be consumed safely. The addition of moringa flour has a significant effect on organoleptic attributes, including color, texture, aroma, and taste, with concentrations of 1%, 2%, and 3% moringa flour, and is still acceptable to panelists.

Advice

Further research is needed on the storage period of beef meatballs at room temperature, considering the addition of different flours.

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