Physical characteristic and antioxidant activity of beef meatballs with addition of *Moringa oleifera* (Lam.) leaves powder

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Abstract. *Moringa oleifera* is one of the herbs that nutritious and rich in bioactive compounds. *Moringa* leaves powder (MLP) is the potential to be applied to processed meat products such as meatball. This study was aimed to evaluate the nutrition content, physical properties, and antioxidant activity of beef meatball added with MLP. There were four levels of treatment employed in the study: Control, which without any addition of MLP; MLP 0.5%, MLP 1%, and MLP 1.5% were meatball added with 0.5, 1, and 1.5% MLP, respectively. All ingredients were well blended to be the dough and boiled at 80°C for 20 min. The results showed that there was no significant difference among treatments for WHC and pH value of meatballs. However, the addition of MLP decreased the cooking loss and enhanced the antioxidant activity of the meatballs. The addition 1.5% declined the lightness, 1% lowered the redness and yellowness value of meatball color. The nutrition content of the meatball was no significant for moisture content, ash, crude protein, and crude fat but increased significantly for crude fibre. It could be concluded that the addition of MLP could improve the antioxidant activity without any changes in WHC, pH value, but by changing the cooking loss and color parameters of the meatballs.

1. Introduction

Meatball is one of the most popular processed meat products consumed by the Indonesian people. Mostly, meatballs are in round form and manufactured from a mixture of meat and permitted food ingredients [1]. Currently, meatballs have developed in many ways of diversification, such as ingredients, serving, name, adding vegetables, and etc. Diversification of processed meatball products aims to improve product quality, increase functional properties, reduce production costs, and increase consumer attractiveness.

Beef meat is the most used in meatball manufacture. Abustam et al (2019) stated that approximately 60% of the beef slaughtered is used for making meatballs [2]. This phenomenon shows that beef meat is a favorite raw material in meatball production. Therefore, studying beef meatballs is influential for developing meatballs in Indonesia.

Woefully, meatballs are perishable so that in the manufacturing need to be added with preservative and antioxidant substances. For safety and healthy reason, nowadays, consumers prefer natural supplementation, such as herbal addition. The use of natural to replace synthetic preservatives is a...
promising technology for increasing the shelf life of meat and meat products. Widely, natural preservatives referring to herbs, fruits, and vegetables both in powders and extracts form have antimicrobial and antioxidant properties.

One source of natural antioxidants that can be incorporated into processed meat is moringa leaves either in powder or extract form [3–5]. Moringa leaves contain ascorbic acid, flavonoids, phenolics, and carotenoids as a source of natural antioxidants [6]. Many researchers have also reported that Moringa oleifera L. is a promising source of natural antioxidants [3]. Oyeyinka and Oyeyinka (2018) reported that Moringa oleifera had been used as a fortificant in many kinds of food [7]. Evivie et al (2015) outlined that the addition of 2% of moringa leaves to the meatball product reduced the panelists’ acceptance, while 1% addition, the panelists’ acceptance was relatively similar to control (without the addition of MLP) [4]. The addition of 0.3% moringa extract to meatball products had the equal oxidation-inhibiting ability as the synthetic antioxidant butylated hydroxyanisole (BHA) [5]. The objective of this study was to evaluate the nutritional content, physical properties, and antioxidant activity of beef meatballs with added moringa leaf powder.

2. Materials and methods

2.1. Materials
The primary ingredients used in this research were beef meat obtained from Antang slaughterhouse, moringa leaf powder made from moringa leaves taken from Gowa regency, tapioca flour, ice cubes, garlic, sodium tripolyphosphate (STTP), salt, pepper, olive oil. The chemicals used were methanol (Merck, Darmstadt, Hesse Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma Aldrich, USA.

2.2. Preparation of leaf powder
The cleaned Moringa leaves were air-dried for a few days then oven-dried for 1 hour at 40°C. The dried leaves were mashed by using a commercial blender and then sieved using a 35 mesh sieve [8]. The powder was kept and for further use.

2.3. Preparation of meatballs
The making of meatballs refers to Abustam et al (2019) method with slight modification [2]. The meat was cut into small pieces and then ground together with salt, ice cubes, and phosphate using a food processor. Furthermore, tapioca flour, flavorings, pepper, garlic, olive oil, and moringa leaf flour were added according to the treatment (0.5%, 1%, 1.5%) and then reground. The homogeneous dough was formed into a round shape and boiled at a temperature of 80°C for 20 minutes. The study was conducted using a completely randomized design with four treatments (0, 0.5, 1, and 1.5% of MLP addition) and five replications.

2.4. Proximate composition
The nutritional compositions were determined by using standard proximate analysis (moisture, protein, ash, fat, and crude fiber content) of the official standard method [9].

2.5. Determination cooking loss and water holding capacity (WHC)
Cooking loss of meatballs was determined by putting 20 g of dough into the plastic and then boiled in a water bath at 80°C for 20 min. Cooking loss was measured based on the ratio of weight lost during cooking and weight uncooked dough [10].

\[
\text{Cooking loss} = \frac{\text{Weight of uncooked dough} - \text{weight of cooked meatball}}{\text{Weight of uncooked dough}} \times 100
\]

The water holding capacity was determined by the centrifugation Jung and Joo’s (2013) method [11]. Ten grams of the sample was put into a 50 mL tube, added with 40 mL of distilled water, and then
incubated in a water bath at 30°C for 30 min. Then it was centrifuged at 3000 rpm for 30 min and re-incubated for 10 min. Then the supernatant was removed and the sample was weighed. The percentage of the WHC was calculated by the formula:

\[
\text{WHC} \, (\%) = \frac{\text{Weight after removing supernatant}}{\text{Weight of sample with water added}} \times 100\%
\]

2.6. The pH value determination
The pH value of meatballs was measured using a pH meter type "BOECO PT380" by piercing the meatballs with an electrode from the pH meter. Prior to use, the pH meter was calibrated at pH 4 and 7.

2.7. Antioxidant activity determination
Sample preparation was carried out by extracting 1 g of sample (meatball) in 5 mL of methanol for 24 hours. A prepared sample of 0.2 mL was reacted with 1.8 mL of 0.06 mMol of DPPH radical solution. Furthermore, the solution was homogenized and incubated at room temperature for 30 min. The absorption value of each concentration was measured at a wavelength of 517 nm using a UV-1800 UV-Vis Spectrophotometer (Shimadzu, USA MFG INC) [12].

\[
\text{Scavenging DPPH} \, (\%) = \frac{(\text{Control Absorbance-Sample Absorbance})}{\text{Control absorbance}} \times 100
\]

2.8. Color measurement
The color (L*, a*, and b*) of the meatballs were measured by using a Color meter test (T135). Prior to use, the instrument was standardized on a white plate (L=94.76, a=-0.795, and b=2.200). Measurements were in triplicate and the average of data was used.

2.9. Statistical analysis
Data obtained were analyzed by analysis of variance (ANOVA) using the general linear models procedure of SAS (version 9.0).

3. Results and discussion
3.1. Nutritional composition
The nutritional composition of the observed meatballs is shown in table 1. Overall, moisture content, ash, crude protein, and crude fat were not affected by the addition of MLP. The MLP remarkably influenced (P<0.05) the crude fiber content only. The increment in crude fiber content was in line with the addition of MLP. This occurrence was quite logical since MLP is a source of crude fiber as just like other leaves and plants. The MLP itself contains 8.29% of fiber content (table 1).

The moisture content obtained in this study was in the range 69.87 to 70.50% which is included in the Indonesia National Standard (SNI 3818: 2014) [13] that allows a max of 70% of moisture content. The fat content (0.71–0.76%) was lowered than the max tolerated by SNI (max 10%) and the protein content (13.90–14.21%) was higher than the min tolerated by the SNI (min 11%). The higher protein content of the meatball added with MLP confirmed Elhadi et al (2017) reported that MLP is rich in protein [14]. The result of this study also exerted the ash content (2.13–2.22%) which was lower than permitted by SNI (max 3%). On the other hand, the nutritional compositional of the meatball was in line with the SNI.
Table 1. Nutritional properties of beef meatballs added moringa leaf powder.

| Formula       | Moisture (%) | Ash (%) | Crude protein (%) | Crude fat (%) | Crude fibre (%) |
|---------------|--------------|---------|-------------------|---------------|-----------------|
| MLP           | 9.65         | 10.88   | 33.37             | 8.55          | 7.49            |
| Control       | 70.50±0.38   | 2.13±0.06b | 14.01±0.17       | 0.76±0.12     | 0.15±0.03b      |
| MLP 0.5%      | 69.90±0.76   | 2.16±0.03b | 14.06±0.50       | 0.71±0.07     | 0.17±0.02b      |
| MLP 1%        | 69.87±0.59   | 2.19±0.01ab | 14.21±0.51       | 0.75±0.03     | 0.23±0.09ab     |
| MLP 1.5%      | 70.26±0.30   | 2.22±0.02a | 13.90±0.29       | 0.73±0.06     | 0.30±0.03a      |

MLP = moringa leaf powder; DM = dry matter; abDifferent superscript in the same column indicates significantly different (P<0.05).

3.2. Water holding capacity, cooking loss and pH

The effect of the addition of MLP on WHC, cooking loss, and pH value of the meatballs is presented in table 1. The result of the study indicated that the addition of MLP exerted a similar WHC value of the meatballs. The WHC value ranged from 31.05 – 33.12 %. This result was contrary to Kenawi and Mohamed [15] that the WHC value without the addition of Moringa oleifera leaf powder was higher than the addition of 1.5%. However, Kenawi and Mohamed (2017) reported that during storage, the loss ability of meatball in holding water decreased significantly in the Control than MLP addition one [15].

Table 2. The water holding capacity, cooking loss and pH value of beef meatballs added with moringa leaf powder.

| Formula       | WHC (%)       | Cooking loss (%) | pH            |
|---------------|---------------|------------------|---------------|
| Control       | 33.12±3.11    | 4.12±0.79a       | 6.20±0.06     |
| MLP 0.5%      | 31.05±2.11    | 2.66±0.89b       | 6.08±0.20     |
| MLP 1%        | 32.39±3.71    | 2.73±0.83b       | 6.11±0.12     |
| MLP 1.5%      | 31.76±2.06    | 2.32±0.78b       | 6.15±0.06     |

MLP = moringa leaf powder; WHC = water holding capacity; aDifferent superscript in the same column indicates no significantly different (P<0.05).

The MLP addition markedly lowered (P<0.05) the cooking loss of the meatballs. Supplementation of MLP 0.5 to 1.5% had no differences effect on cooking loss among them but significantly different from control. The decreasing cooking loss of the meatballs added with MLP indicates that MLP plays a role in binding water. Abustam et al (2020) reported that MLP has a property in binding the water of processed meat products so that declining cooking loss of the meatballs [10]. This study also confirmed Ibrahim et al (2017) stated that the percentage of cooking loss in meat-rice kofta with the addition of MLP decreased from the control sample [16]. Theoretically, increasing the binding capacity of raw meat during cooking will reduce cooking losses.

The result of this study indicated that the pH value of control meatballs and those added with MLP was not significantly different. The pH value obtained in this study ranged from 6.0 to 6.20. This study also confirmed Abustam et al (2020) that the addition of MLP could not change the pH of meatballs [10]. These results showed that the addition of MLP had no effect on the physicochemical properties of meatballs. In contrast, Elhadi et al (2017) reported that the incorporation of MLP to chicken patty declined the pH value. This different result most probably caused by different concentrations of MLP added to the products. Elhadi et al (2017) added MLP higher than this study [14]. However, other reports indicated that the pH of meat products containing moringa leaf and seed extract increase. It could be the herb added was due to in extract [17,18].

3.3. Antioxidant activity and color

The antioxidant activity of beef meatballs was significantly increased with an elevation of every 0.5% addition of MLP, as shown in table 3. It can be concluded that the higher the level of MLP added, the higher the antioxidant activity. The antioxidant activity is a capability of meatballs in scavenging DPPH free radicals. The higher capability of this means the antioxidant content of the meatballs is adequate.
The higher the antioxidant activity of the meatball was most probably due to MLP added. Many studies reported that moringa leaves consist of phytochemicals such as flavonoids, saponins cytokinins, acid-caffeoylquinat and contain unsaturated fatty acids such as linoleic (omega 6) and alfalinolenat (omega-3) [19] and has very high antioxidant activity [20]. Other researchers investigated that the antioxidant activity of patties added with moringa increased [21]. This study confirmed to other researchers that higher antioxidant activity of the moringa leaves proved that this kind of leaves can be considered as a source of natural antioxidants [22].

Table 3. The antioxidant activity and L*, a*, b* values of beef meatballs added moringa leaf powder.

| Formula          | DPPH (%) | L*       | a*       | b*       |
|------------------|----------|----------|----------|----------|
| Control          | 25.03±1.15<sup>d</sup> | 50.68±0.91<sup>a</sup> | 11.51±1.57<sup>a</sup> | 12.60±0.83<sup>c</sup> |
| MLP 0.5%         | 29.69±1.66<sup>c</sup> | 51.26±2.07<sup>a</sup> | 6.47±1.92<sup>b</sup> | 15.86±1.82<sup>b</sup> |
| MLP 1%           | 32.48±1.02<sup>b</sup> | 51.67±4.33<sup>a</sup> | 4.04±0.90<sup>c</sup> | 18.43±1.18<sup>a</sup> |
| MLP 1.5%         | 34.68±0.97<sup>a</sup> | 45.63±1.20<sup>b</sup> | 2.58±0.82<sup>c</sup> | 17.54±0.88<sup>a</sup> |

MLP = moringa leaf powder; different superscript in the same column indicates significantly different (P<0.05).

The color parameters of the study are shown in table 3. The addition of 1.5% MLP reduced the lightness (L*) of the meatballs. It most probably caused by the addition of 1.5% enhanced the dark color of the meatball. This result was in reverse to the research of Al-Juhaimi et al (2016) reported that the lightness, as measured by the L* value of the patties, was increased with the MLP level and the lowest lightness value was obtained in non-formulated beef patties [17]. Other studies confirmed that natural antioxidants did not cause a decrease, but rather a small increase in the L* value of the sample [23]. The different result of these was probably due to a different kind of product. However, this study resulted that the add up to 1% did not change the lightness of the meatballs.

The redness of the meatball notably decreased by the increase of MLP level. This result was in accordance with the research of Ergezer et al (2018) stated that the redness of the sample added with natural antioxidants could not maintain the redness of the product. The low redness of the meatball could be the MLP contained a greenish-brownish pigment, which may have led to reduced redness of the meatball [23].

On the other side, the value of b* (yellowness) increased by the increase of MLP level. This result was also in accordance with Al-Juhaimi et al (2016) that reported the increased yellowness in the moringa seed flour formulation was probably due to the presence of carotenoid pigments in the moringa seed flour [17]. Al-Juhaimi et al (2018) also reported that the addition of argel leaf powder on camel patty increased the yellowness than the control patty [24].

4. Conclusion
The addition of moringa leaf powder could enhance the antioxidant activity and crude fiber of the meatball with no shifting of WHC and pH value. These characteristics of the meatball meet the requirement of healthy food.

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