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Analysis Proximate and Iron Content in Beef Meatball Substituted by Oyster Mushrooms (*Pleurotus ostreatus*)

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ABSTRACT
Meatballs were generally made using beef and starch flour, but the expensive price of beef makes meatballs a food that is difficult to afford for people with low economic levels. The selection of oyster mushrooms as an ingredient in the manufacture of meatballs meant to achieve higher nutritional content with an affordable price. This study aimed to find out the nutritional value and iron content of oyster mushroom meatballs. This study used an experimental method with 4 formulations that include variations in the concentration of beef: oyster mushrooms K 100%:0%, F1 75%:25%, F3 25%:75%, F3 0%:100%. This study used a complete randomized design method. Data analysis was used ONE WAY ANOVA and advanced analysis with Duncan multiple test range. ONE WAY ANOVA analysis showed that there were differences in nutritional value and iron content in four formulations in beef meatball substituted by oyster mushroom. Advanced analysis with Duncan multiple test range showed a noticeable difference in the levels of protein, water, ash, fat, carbohydrates, and iron in beef meatball substituted by oyster mushroom. The best formulation with nutritional and iron content was the control formulation with a 100% proportion of beef, with protein 11.04%, water 64.23%, ash 1.71%, fat 1.5%, carbohydrates 21.50%, and iron 67.72 mg. The study concluded that the best formulation with the highest nutritional and iron content was in the control treatment.

INTRODUCTION

Meat is a food with high nutritional value. Among the nutrients found in meat are protein, iron, and zinc (Yeung & Laquatra, 2003), which are essential for human growth. However, the current high price of meat has led to low meat consumption among the Indonesian population. Compared to global consumption, the average meat consumption in Indonesia in 2017 was only 1.8 kg, which is below the global average of 4.6 kg. Meatballs are a protein-rich food that is familiar to people from all walks of life and is very popular among Indonesians. Their savory flavor and chewy texture (thanks to their beef base) make meatballs a favorite among the public. However, meatballs in Indonesia are still often made with beef, which is unaffordable for people with low incomes. Therefore, there is a need for an alternative high-protein ingredient that is more affordable. Oyster mushrooms are edible and highly nutritious; 100 grams of oyster mushrooms contain 1.9 grams of protein, 0.1 grams of fat, 5.5 grams of carbohydrates, 0.7 mg of iron, and 9 mg of calcium. Additionally, oyster mushrooms are readily available in markets because they grow in nearly every region of Indonesia's tropical climate. Oyster mushrooms are well-known for their many health benefits and can serve as an alternative to animal-based protein sources due to their high plant-based protein content. The lower price of oyster mushrooms helps reduce expenses for people who cannot afford meat. Furthermore, spending on high-protein side dishes will be reduced, allowing people to purchase a wider variety of food items. As Allah SWT commands in the Quran, humans are instructed to be mindful of their diet and to consume a variety of foods to meet their needs for carbohydrates, protein, fat, and other nutrients. Allah has also created a wide variety of food sources so that humans can meet their needs from various sources (ranging from plant-based to animal-based), and from affordable to expensive. It is hoped that oyster mushroom meatballs will be enjoyed by the Indonesian public

because they are high in protein, just like traditional meatballs. The protein content in oyster mushroom meatballs can be determined through proximate analysis.

MATERIALS AND METHODS

The method used in this study was an experimental method, which involved making beef meatballs with the addition of oyster mushrooms at different concentrations. A completely randomized design was used, with four treatments, as follows:

Control = beef (100%)
F1 = beef : oyster mushrooms (75%:25%)
F2 = beef : oyster mushrooms (25%:75%)
F3 = oyster mushrooms (100%)

The ingredients used to prepare the beef meatball samples with oyster mushroom substitution were beef, oyster mushrooms, tapioca flour, red onion, garlic, egg whites, ice cubes, and cooking oil.

Table 1. Ingredient composition of the meatball samples (g)

Formula	Control	F1	F2	F3
Beef	250	187,5	62,5	0
Oyster mushrooms	0	62,5	187,5	250
Table salt	5	5	5	5
Pepper	5	5	5	5
Garlic	12	12	12	12
Starch	40	40	40	40
Flour	15	15	15	15
Egg whites	10	10	10	10

Formulations: control (100% beef); F1 (75% beef and 25% oyster mushrooms); F2 (25% beef and 75% oyster mushrooms); F3 (100% oyster mushrooms).

The materials used for the proximate analysis and iron content tests are distilled water, sulfuric acid (H_2SO_4), 0.1N hydrochloric acid (HCl), 30% sodium hydroxide (NaOH), and boric acid.

The equipment used for sample preparation includes a plastic basin, a tablespoon, a stirrer, a digital scale, a measuring cup, a stove, a blender, and a knife. The equipment used in the proximate analysis included a furnace, a Memmert oven, a Soxhlet apparatus, a Kjeldahl apparatus, an SSA (Shimadzu 1900), glassware, a burette, an electric stove, an analytical balance, a spatula, and beakers.

This study was conducted at the Integrated Laboratory of Darussalam Gontor University and ran from November 2021 to March 2022. Data collection techniques in this study included proximate analysis (protein, moisture, ash, fat, and carbohydrates) and iron content testing. The data obtained will be analyzed using a one-way ANOVA followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

This study assessed the proximate composition and iron content of beef meatballs substituted with oyster mushrooms. The results of the proximate and iron analyses are presented in Table 2.

Table 2. Proximate composition of the meatball formulations

Analysis	Group	Value	Indonesian standard for combination meatballs (% w/w)
Protein content	control	11.04^{d}	>8.0
	F1	9.56^{c}	
	F2	6.61^{b}	
	F3	3.42^{a}	
Moisture content	control	64.23^{a}	<70.0
	F1	68.02^{b}	
	F2	70.90^{c}	
	F3	74.54^{d}	
Ash content	control	1.71^{d}	<3.0
	F1	1.40^{c}	
	F2	1.22^{b}	
	F3	1.06^{a}	
Fat content	control	1.50^{d}	<10
	F1	1.10^{c}	
	F2	0.90^{b}	

	F3	0.60[a]

Different superscript letters indicate statistically significant differences ($p < 0.05$). Control = 100% beef; F1 = 75% beef and 25% oyster mushrooms; F2 = 25% beef and 75% oyster mushrooms; F3 = 100% oyster mushrooms.

Protein content

Protein is a macronutrient that primarily supports tissue growth and the formation of biomolecules rather than serving as an energy source. However, when energy intake is insufficient, protein can also be used as an energy source (Yeung & Laquatra, 2003). The highest protein contents were reported for the control and F1 formulations, whereas the lowest values were observed in F2 and F3. This pattern suggests that protein content increased with the proportion of beef. Beef has been reported to contain approximately 18–26 g protein per 100 g (Suryani et al., 2014), while the reported protein content of oyster mushrooms ranges from 10.5% to 30.4% per 100 g. According to the Indonesian National Standard cited in the manuscript (SNI 01-3818-1995), combination meatballs should contain at least 8.0% protein (w/w). The control and F1 formulations met this criterion, whereas F2 and F3 did not. The manuscript attributes the lower protein levels in the mushroom-rich formulations partly to changes during boiling and cooking, including protein denaturation and the loss of water-soluble compounds into the cooking water (Sutikarini et al., 2015).

A previous study reported the highest protein content in oyster-mushroom meatballs made with 100 g beef (62.76%) and the lowest in meatballs made with 100 g oyster mushrooms (32.43%) (Suryani et al., 2014). These values and units should be checked against the cited source before publication. One-way ANOVA for protein content was reported as $p = 0.000$, indicating a statistically significant difference among the formulations. For reporting, this should generally be expressed as $p < 0.001$.

Moisture content

Although water does not provide energy like other nutrients, it is essential for biochemical processes in living organisms and is a major component of food (A. Rohman & Sumantri, 2013). Moisture content affects food quality, freshness, and shelf life; products with higher moisture content may be more perishable (Fillaili et al., 2020). As shown in Table 2, moisture content increased as the proportion of oyster mushrooms increased. The highest moisture content was observed in F3, which contained 100% oyster mushrooms (74.54%). Moisture content is influenced by the raw materials used. The manuscript suggests that oyster mushrooms have a greater water-holding capacity than beef, which may explain the higher moisture content in formulations containing more mushrooms (Siska et al., 2018). Moisture content was 64.23% in the control, 68.02% in F1, 70.90% in F2, and 74.54% in F3. Based on the maximum moisture criterion of 70% cited for SNI 01-3818-1995, the control and F1 met the criterion, while F2 and F3 exceeded it. The manuscript reports a statistically significant difference in moisture content among formulations ($p < 0.001$). The reported one-way ANOVA result for moisture content was $p = 0.000$, indicating a statistically significant difference among the formulations; this should be reported as $p < 0.001$.

Ash content

Ash content represents the inorganic residue, including minerals, remaining after food is incinerated. The ash content of a food provides an estimate of its total mineral content, although the mineral composition depends on the ingredients and the ashing method (Rohman, 2013). The highest ash content was observed in the control formulation, containing 100% beef, at 1.71%. Ash content decreased as the proportion of oyster mushrooms increased. Conversely, formulations containing a greater proportion of beef had higher ash content. This pattern is consistent with a previous report that the highest ash content in square oyster-mushroom meatballs occurred in a formulation containing 37.5 g beef and 12.5 g oyster mushrooms (Sri, 2019). Boiled oyster mushrooms have been reported to contain 2.89% ash, while beef has been reported to contain 5.40% (Ma'rifah & Sari, 2017). All four formulations were below the maximum ash content of 3.0% (w/w) cited for combination meatballs. The reported ash contents were 1.71% for the control, 1.40% for F1, 1.22% for F2, and 1.06% for F3 (Badan Standarisasi Nasional, 2014). One-way ANOVA indicated a statistically significant difference in ash content among the beef meatball formulations containing different proportions of oyster mushrooms (reported $p = 0.000$; report as $p < 0.001$).

Fat content

Fat is an energy-dense nutrient and, like other lipids, is soluble in organic solvents but not in water (Tiien Sofiati et al., 2020). Dietary fat provides essential fatty acids, although excessive consumption may be associated with adverse health outcomes, including dyslipidemia (Pratiwi et al., 2020). The highest fat content was observed in the control formulation (100% beef) at 1.50%. Fat content decreased as the proportion of oyster mushrooms increased. The fat in these meatballs likely reflects the natural fat content of the beef and oyster mushrooms (Abdul, 2014). The authors attribute the higher fat content in beef-rich formulations to the higher fat content of beef compared with oyster mushrooms. The manuscript cites fat contents of 25% for beef

and 1.6% for oyster mushrooms (Falahudin et al., 2017); these values should be verified against the cited source. Oyster mushrooms are also described as a low-fat food, and their fiber may affect the measured fat content. A previous study reported that adding oyster mushrooms to meatballs affected the final fat content, with higher mushroom proportions associated with lower fat levels (Sri, 2019).

All four formulations were below the maximum fat content of 10% (w/w) cited for combination meatballs. The reported values were 1.50% for the control, 1.10% for F1, 0.90% for F2, and 0.60% for F3. Note that some values differ slightly from those stated elsewhere in the manuscript and should be reconciled with the original data. One-way ANOVA indicated a statistically significant difference in fat content among the four formulations (reported $p = 0.000$; report as $p < 0.001$).

Carbohydrate content

Carbohydrates are an important dietary energy source and can provide a substantial proportion of daily energy requirements (Sifiati et al., 2020). They also have important functional roles in food processing and can contribute sweetness, particularly in the case of mono- and disaccharides (Lamadjido, 2019). Table 3 shows that carbohydrate content varied numerically among the four formulations. The highest value was reported for the control (21.50%), followed by F3 (20.32%), F2 (20.30%), and F1 (19.90%). The manuscript suggests that the higher carbohydrate value in the control may reflect its overall proximate composition. Carbohydrate content was calculated by difference and therefore depends on the measured values of the other proximate components (Sri, 2019). The reported one-way ANOVA result was $p = 0.075$, indicating no statistically significant difference among formulations.

Table 3. Carbohydrate content (g/100 g)

No.	Analysis	Group	Value
1	Carbohydrate content	control	21.50^{b}
2		F1	19.90^{a}
3		F2	20.30^{ab}
4		F3	20.32^{ab}

Different superscript letters indicate statistically significant differences ($p < 0.05$). Control = 100% beef; F1 = 75% beef and 25% oyster mushrooms; F2 = 25% beef and 75% oyster mushrooms; F3 = 100% oyster mushrooms.

Iron content

Iron is an essential micronutrient involved in hemoglobin formation. Approximately two-thirds of the body's iron is found in hemoglobin. Inadequate iron intake can lead to anemia, and prolonged depletion of iron stores can reduce serum ferritin levels (Anggraini et al., 2015). Beef and oyster mushrooms are both dietary sources of iron. The manuscript notes that oyster mushrooms contain iron in addition to protein (Hayyuningsih et al., 2009). As shown in Table 4, the highest iron content was observed in the control formulation containing 100% beef. This suggests that the proportion of beef affected the iron content of the meatballs. The manuscript cites iron contents of 2.6 mg for beef and 0.7 mg for oyster mushrooms (Salman et al., 2018). The authors also note that the control had the highest ash content, which they interpret as consistent with a higher total mineral content, including iron. One-way ANOVA indicated a statistically significant difference in iron content among the four formulations (reported $p = 0.000$; report as $p < 0.001$).

Table 4. Iron content (mg/100 g)

No.	Analysis	Group	Value
1	Iron content	control	67.72^{d}
2		F1	60.55^{c}
3		F2	33.18^{b}
4		F3	23.40^{a}

Different superscript letters indicate statistically significant differences ($p < 0.05$). Control = 100% beef; F1 = 75% beef and 25% oyster mushrooms; F2 = 25% beef and 75% oyster mushrooms; F3 = 100% oyster mushrooms.

CONCLUSIONS

Based on the proximate analysis and iron measurements, the study shows that the protein content was 11.04% in the control, 9.56% in F1, 6.61% in F2, and 3.42% in F3. Moisture content was 64.23%, 68.02%, 70.90%, and 74.54%, respectively. Ash content was 1.71%, 1.40%, 1.22%, and 1.06%, respectively. Fat content was reported as 1.50%, 1.10%, 0.90%, and 0.60%, respectively. Carbohydrate content was 21.50% in the control, 19.90% in F1, 20.30% in F2, and 20.32% in F3. Iron content was reported as 67.72 mg in the control, 60.55 mg in F1, 33.18 mg in F2, and 23.40 mg in F3. The study states that the best proximate and iron results were obtained in F2 because it met the Indonesian National Standard for combination meatballs

(Badan Standarisasi Nasional, 2014). However, this conclusion appears inconsistent with the values presented in the results tables and should be verified against the intended criteria and original data.

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