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Nutrient and Proximate Composition Analysis of Meatballs Formulated with Edible Mushrooms

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The increasing demand for sustainable and healthier food alternatives has led to the exploration of plant-based ingredients in food formulations. This study investigates the incorporation of *Pleurotus sajor-caju* mushroom into meatballs to reduce meat consumption while maintaining acceptable nutritional and sensory qualities. Three formulations with varying meat-to-mushroom ratios as Sample 1 (74:10), Sample 2 (64:20), and Sample 3 (54:30), were developed. Nutritional and physical analyses were tested to evaluate the influence of mushroom incorporation of the meatballs. The findings revealed a significant reduction in fat content as the mushroom incorporation percentage increased. Fat content decreased from 32.75% in the formulation of Sample 1 to 10.25% in the formulation of Sample 3, indicating the potential of mushroom-based meatballs as a low-fat alternative. Improvements in the functional properties of the meatballs were also observed. Water holding capacity (WHC) increased from 8.5% to 15%, while moisture content rose from 67.65% to 73.42% with higher mushroom ratios. These improvements resulted in softer and juicier textures, comparable to conventional meatballs. This research demonstrates that *Pleurotus sajor-caju* can be effectively used to produce healthier, lower-fat, and more environmentally sustainable meatball alternatives without compromising texture or flavor.

Keywords: Mushroom, *Pleurotus sajor-caju*, meatball, proximate composition, nutrient

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Few studies have been conducted on the types of mushrooms that are halal and suitable for fat replacement in meatballs. Few mushrooms suitable for this are Shiitake mushroom (*Lentinula edodes*), brown button mushroom (*Agaricus bisporus*), but they require mixing for a longer time to produce high-quality meatballs [1], and king oyster mushroom, which, however, reduced the texture quality of the reduced-fat meatballs [2]. Compared to regular meatballs, mushrooms require less land to grow and can even be grown indoors, compared to livestock for meatballs, and in addition, mushrooms leave smaller carbon footprints compared to livestock production, as they significantly contribute to greenhouse gas emissions. Overall, mushrooms are more sustainable compared to meat as they have a lower environmental impact [3]. Consumer preferences also contribute to how widely mushroom meatballs can be adopted compared to the traditional options.

Since conventional meat production emits such a significant environmental impact, there is a growing need for better alternatives, which makes mushrooms a suitable meat substitute due to their nutritional content, versatility and lower environmental impact compared to meat production. This project is conducted to address these challenges by exploring the varieties of mushroom available and their suitability in creating delectable and satisfying Halal mushroom meatballs to promote more sustainable food choices in Halal dietary plan specifically towards the of *Pleurotus sajor-caju* incorporation into meatballs to reduce meat consumption by formulating mushroom meatballs as a substitute for conventional meat and determining the nutritional content of mushroom meatballs which was compared with conventional meatballs. The meatball processing was carried out based on the method described by [2]. Pleurotus sajor-caju mushroom *(Pleurotus sajor-caju)* will be used in this study to produce Halal meatballs. Three formulations will be carried out with the addition of mushrooms and other ingredients like ice water, sugar, black pepper, corn flour, sugar, Sodium tripolyphosphate and monosodium glutamate. This mushroom will be compared with control and commercial meat-based meatballs. Chemical and physical analysis will be conducted at the Faculty of Industrial Science and Technology (FIST), Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA). Chemical analysis performed will include nutrient analysis (moisture content, protein, fat,

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carbohydrate and salt) whereas physical analysis involves texture profile analysis (firmness) and water holding capacity (WHC).

EXPERIMENTAL

Chemicals and Materials

Beef meat, commercial meat-based meatballs were bought from the Tunas Manja Group Market at Gambang, Pahang, Malaysia while the *Pleurotus sajor-caju* mushrooms were collected at the mushroom house at the Universiti Malaysia Pahang Al-Sultan Abdullah, Malaysia. Ice water, hot water, salt, black pepper, sodium tripolyphosphate (STTP), corn flour, monosodium glutamate (MSG), onion powder, sugar, sodium carbonate, hydrochloric acid (HCl), sodium hydroxide (NaOH) Bovine Serum Albumin (BSA), petroleum spirit, n-hexane, sulphuric acid, Folin & Ciocalteu's Phenol reagent, Bradford reagent, phosphate-buffered saline were materials used in this study.

Preparation and Formulation of Mushroom Meatballs

The meatballs consisted of 74% minced meat mixed with 10% *Pleurotus sajor-caju* mushroom, 8.7% ice water, 4.2% corn flour, 1.0% salt, 0.7% onion powder, 0.6% sugar, 0.5% sodium tripolyphosphate (STPP), 0.2% black pepper and 0.1% monosodium glutamate (MSG). The meatballs were produced following the method described by[1] with slight modification. All the ingredients were mixed for 15 min in a bowl until homogenized. Afterwards, salt and STTP were added, followed by ice water to control the temperature. The round meatballs were formed manually with a weight of 15±0.5 g each and were left for 20 min to set. The set meatballs were further boiled in hot water of approximately 80 °C for 25 min. Then, the excessive water was drained out and the meatballs were left to cool at room temperature, followed by storage in a freezer (-18 °C) for further analysis. Three formulations (**Table 1**) were carried out in this experiment.

Moisture Content

Moisture content of the meatballs was determined by comparing the weight of the sample before drying and after oven drying at 100°C overnight [4] and was calculated based on the weight loss upon drying on Equation 1:

$$Moisture\ content\ (\%) = \frac{Wt\ before\ (g) - Wt\ after(g)}{Wbefore\ (g)} \times 100\% \qquad \text{Eq 1}$$

Table 1. Formulation of mushroom meatballs.

Ingredients	Percentage (%)		
	A	B	C
Minced meat	74%	64%	54%
Pleurotus sajor-caju mushroom	10%	20%	30%
Corn flour	4.2%	4.2%	4.2%
Salt	1.0%	1.0%	1.0%
Onion powder	0.7%	0.7%	0.7%
Sugar	0.6%	0.6%	0.6%
STTP	0.5%	0.5%	0.5%
Black pepper	0.2%	0.2%	0.2%
MSG	0.1%	0.1%	0.1%

Protein Content

For the extraction of protein, four grams of the minced meatball were homogenized with 80 ml of ice-cooled buffered solution in a homogenizing tube placed in ice for 4 min. The homogenate solutions were centrifuged using a high-speed centrifuge (Centrifuge Kubota 3740, Kubota Corp., Japan) at 10,000 g for 1 h at 4 °C. After centrifugation, the protein isolate obtained from the supernatants was used for the determination of protein concentration by the Bradford method [5]. The Bradford protein quantification method consists of a series of procedures that must be followed to guarantee precise measurement. First, samples and protein standards are made. Bovine serum albumin (BSA) mixed with Phosphate-buffered saline (PBS) was used as the stock solution and standard solution. The samples were then mixed with the Bradford reagent. When this reagent interacts with the supernatants, a blue hue is produced. After that, the samples were incubated for 10 minutes to allow for complete colour development. Afterwards, the sample absorbance will be measured at 595 nm using a UV-VIS spectrophotometer (Genesis 150 UV-VIS). Bovine serum albumin concentration vs absorbance calibration curve to analyse the protein concentration by comparing the absorbance to a standard curve from the protein standards of meatballs with the samples' absorbance.

Fat Content

Fat content analysis was conducted by using the Soxhlet extraction method. All the glassware that was used is rinsed with n-hexane, drained and dried in the oven at 102 ºC for 30 min. and cooled in a desiccator. A piece of cotton wool was placed at the bottom of a 100 mL beaker. A plug of cotton wool was put in the bottom of the extraction thimble. The thimble will stand in the beaker. 5 g of the sample was dried in the oven at 102ºC for 5 h in the thimble. The sample cooled down in the desiccator. A piece of wool from the beaker was put into the thimble. The thimble was inserted into the Soxhlet liquid/solid extractor. 150 mL of n-hexane was added to a 500 mL round-bottom flask. The extraction unit was assembled over an electric heating mantle. The solvent in the flask was heated to a boil. The heat source was adjusted so that the solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second. The extraction was done in stages. After 4 h, the heat source was removed, and the solvent was drained. The thimble was removed from the extractor and the sample was transferred to a 200 mL beaker. The sample was broken by using the glass rod and returned afterwards into the thimble. The thimble in the extractor was replaced. The beaker was rinsed with 150 mL n-hexane and the rinsing was poured into the extract. Extraction was continued for another 2 h. Afterwards, the fat was extracted using a rotavapor. The flask and the contents afterwards were weighed, and the fat content was calculated by using equation 2. The experiments were repeated with all three formulations.

$$Fat\ (\%) = [\![[W]]\!]_{flask+fat}\ (g) - W_{empty\ flask}\ (g)] \times \frac{100}{Weight\ of\ sample} \quad \text{Eq 2}$$

Carbohydrates Content

Carbohydrate analysis was determined by using a UV-VIS spectrophotometer, a method conducted by [6]. To guarantee consistency, the operation was started by homogenizing the meat sample. The homogenized sample was weighed precisely to the extent of 5 g. Afterwards, the sample was heated to a specific temperature and subjected to acid hydrolysis, which split complex polysaccharides into their monosaccharide constituents. Hydrochloric acid (HCl) was added. After hydrolysis, sodium hydroxide (NaOH) was added to the solution to neutralize the pH. After neutralization, the mixture was filtered to eliminate any remaining particles and leave only the liquid that contains carbohydrates. After that, the filtrate's monosaccharides were derivatized to improve their detection in the UV-VIS spectrophotometer. Adding a derivatizing agent, which reacts with the monosaccharides to generate detectable derivatives, is a step in the derivatization process. After the sample has been derivatized, it was added to the UV-VIS spectrophotometer (Genesis 150 UV-VIS) and the absorbance was measured at 595 nm. 5% Phenol mixed with 5 mL of sulphuric acid was used as the standard solution. The standard solution concentration vs absorbance calibration curve was used to analyse the carbohydrate concentration by comparing the absorbance to a standard curve from the carbohydrate standards of meatballs with the samples' absorbance.

Water Holding Capacity

By using the method described by [7], the water holding capacity (WHC) of the mushroom meatballs was measured by cutting the sample in half after being thawed for 30 min at room temperature. The sample was then trimmed carefully until the final weight of 2 g before being wrapped inside a filter paper, Whatman no. 1 (601), size 125 mm and loaded in a 50 mL centrifuge tube. Afterwards, the sample was centrifuged at 5000 rpm, 25ºC for 15 minutes using a high-speed centrifuge (Centrifuge Kubota 3740, Kubota Corp., Japan). The weight of the mushroom meatballs before and after was recorded and calculated by using Equation 3:

$$WHC\ (\%) = \frac{Wt\ before\ (g) - Wt\ after(g)}{Wbefore\ (g)} \times 100\% \quad \text{Eq 3}$$

Data Analysis

Statistical analysis was conducted using a one-way ANOVA to compare the mean in proximate composition. The level of significance was set at $p < 0.05$ to identify the significance difference among the three replicates.

RESULTS AND DISCUSSION

Moisture Content

Moisture content contributes to the texture and sensory qualities of the meatballs. Thus, higher moisture content improved the qualities of the meatballs, such as the juiciness, while reducing the reliance on fat while making it a healthier option and enhancing the sustainability, however, certain aspects must be considered as well in creating plant-based alternatives, such as shelf life and microbial stability [8]. **Table 2** shows the moisture content percentages calculated using equation 1 for three samples with varying meat and mushroom percentages. Sample 1 contains 74% meat and 10% mushroom, has the lowest moisture content at 67.65% compared to all samples, reflecting the influences of the highest meat percentage of all samples since meat generally contains less water compared to mushrooms. This can be further observed in Sample 2 as the moisture content increases to 69.62% with a mixture of 64% meat and 20% mushroom.

Increasing the mushroom percentage increases water retention thus increasing its moisture content, balancing with the drier nature of the meat and this trend is further confirmed with the moisture content of Sample 3 at 73.42 % with lowest meat percentage (54%) and highest mushroom percentage (30%) compared to all samples. Mushroom compositions are rich in their ability to retain moisture during processing due to the presence of polysaccharides such as beta-glucan which are hydrophilic in nature thus help reduce moisture loss which maintain the meatballs juiciness even with reduced meat percentage as meat inherent lower moisture content especially at high temperature [9].

Protein Content

In the human diet, meat and dairy are significant sources of protein, vitamins, and minerals. Given that exogenous nutritional supplements can include vitamin A, calcium, and phosphorus, which are found in milk, as well as B vitamins and iron, which are found in meat. The primary criteria for assessing the nutritional value of meat and milk substitute proteins are the protein content, amino acid composition, and protein digestibility, as well as any fortifiers added to animal substitute products. Meat is typically rich in protein and since different plant protein sources must be combined to replicate the protein found in meat, it is challenging to match the amino acid levels of meat with plant-based meat substitutes. Even when it is, the bioavailability of plant protein is significantly lower than that of its animal counterpart [10]. The protein content of meat is typically about 20%, while protein content of *Pleurotus sajor-caju* mushroom varies depending on the growing conditions and the preparation. For example, fresh mushroom contains 4% (3.5 g per 100g) of protein on average, while dried mushrooms contain higher protein concentrations ranging from 20-40% (30g per 100 g) of their dry weight due to the removal of water [11].

Table 2. Moisture content percentage.

Sample	Weigh before (g)	Weigh after (g)	Moisture content (%)
1	2.38	0.77	67.65 ± 1.83
2	2.38	0.72	69.62 ± 1.06
3	2.38	0.63	73.42 ± 2.11

Table 3. Protein content absorbance and concentration of standard solution.

Concentration (μg/mL)	Absorbance
100	1.390
200	1.391
400	1.409
600	1.420
800	1.439
1000	1.448

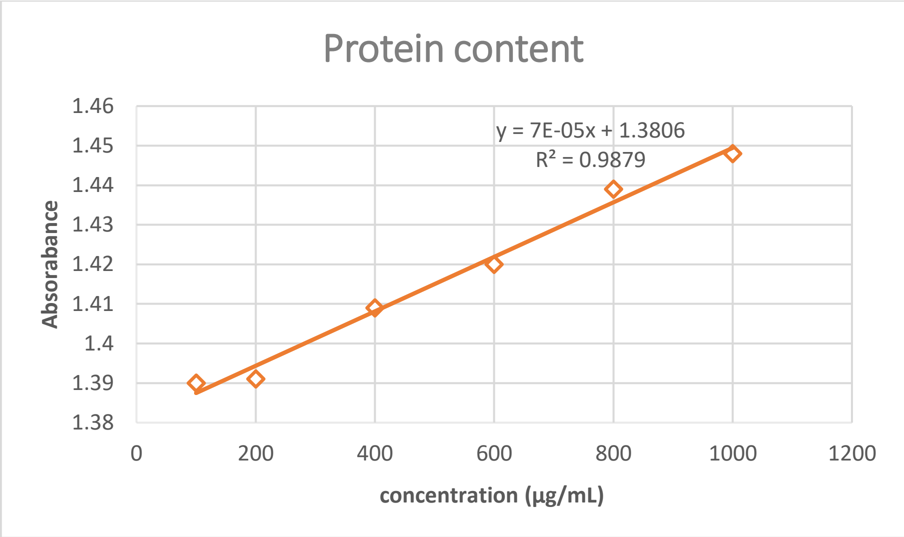

Figure 1. Protein content standard solution calibration curve.

Table 4. Sample protein content absorbance and concentration.

Sample	Absorbance	Concentration (mg/L)
1	1.542	2.3057
2	1.411	0.4343
3	1.359	-0.3086

The absorbance of the standard solution (**Figure 1**) can be seen as the concentration is directly proportional to the absorbance of the standard solution, indicating a near-plateau of the relationship as the concentration increases. This shows that the accuracy of the standard curve is almost accurate since the coefficient of determination is nearing 1 (0.9879). The concentration of the protein in the sample (**Table 4**) was calculated based on an equation obtained from the graph (y = 7e-05x + 1.3806) shows a variation according to their absorbance by inserting absorbance as y and concentration as x. However, certain inconsistencies are observed, such as Sample 1 (74% meat content), concentration is 2.3057 mg/mL at 1.542 nm. Sample 1 exhibits the highest concentration of protein content as expected, which corresponds to the fact that Sample 1 contains the highest meat percentage compared to the rest of the samples. Sample 2 (64% meat) shows a concentration of 0.4343 mg/mL at an absorbance of 1.411. The concentration of the protein in Sample 2 drops significantly, reflecting the reduced meat percentage and lower protein content compared to Sample 1.

However, Sample 3 shows a negative concentration, which is -0.3086 mg/mL at 1.359 nm. The negative value indicates that the protein concentration was too low to be detected within the visible light [6]. Despite the negative value obtained, all three sample protein concentrations are positively correlated with the meat percentage, as the meat percentage was reduced, so was the protein concentration, even with the increasing mushroom concentration. This is due to the characteristics of mushrooms, as mushrooms primarily dilute the protein content, as they are lower in percentage of protein compared to meat, even with the presence of similar amino acids in meat, such as lysine [6]. However, even with the overall lower protein content in mushroom-based meatballs compared to traditional meatballs, the addition of mushrooms leverages the functional benefits, such as improvement of water holding capacity and sustainability, thus can meet the sustainability goals and consumer acceptance.

Fat Content

Table 5 shows the percentage of fat for the three samples in mushroom meatballs with different content of meat, which are 74%, 64% and 54% respectively, that were extracted by using Soxhlet extraction. The results suggest insight into how the proportion of meat and mushrooms influences the fat content of the meatballs. Sample 1 contains 32.75% fat percentage which is the highest fat percentage compared to other samples thus correspond positively to the fact that it aligns with the expectation of higher meat percentage that contributes to more fat since red meat particularly contains a significant amount of fat as meat contains visible fats, both adipose tissue and intramuscular fat which will be retained in the meatball's formulation.

Table 5. Fat content percentage.

Sample	Weigh of empty flask with fat (g)	Weigh of empty flask (g)	Fat (%)
1	181.81	180.5	32.75 ± 0.72
2	181.58	180.5	27.00 ± 1.05
3	180.91	180.5	10.25 ± 0.92

This also indicates that the incorporation of mushrooms in the meatballs dilutes the percentage of fat as mushrooms contain negligible fat content, thus reducing the fat and increasing water and dietary fibre percentage, which can be observed in Sample 2 (27 %) and Sample 3 (10.25%) fat percentage. By reducing the meat percentage in Samples 2 and 3 to 64% and 54% and increasing the mushroom percentage to 20 % and 30%, respectively, the fat percentage shows a notable decrease in each sample, which reflects one of the mushroom properties, which is low in fat (0.5 g per 100 g). This trend suggests that the incorporation of mushrooms in the mixture dilutes the fat present in the meat and emphasizes their role as a low-fat ingredient in the formulation, which aligns with the goals in creating healthier options for consumers while also providing more sustainable meatball alternatives. However, Sample 2 was the optimum fat percentage since at 27%, it achieves a balance between the reduced fat and the flavor, making it suitable for fat-reduction goals. The results clearly show a negative correlation between the meatballs' fat percentage and mushroom concentration. By successfully lowering the fat content, mushrooms help create healthier formulations without sacrificing their functionality.

Carbohydrate Content

Based on the calibration curve of the standard solution in **Figure 2** and **Table 6**, carbohydrate content increases as the mushroom percentage increases and the meat percentage decreases in the sample's formulation. Mushroom is a natural source of carbohydrates that contributes positively to the functional properties of meatballs. The standard curve demonstrates a positive correlation between the absorbance and carbohydrate concentration as the concentration of carbohydrate content increases alongside the increase in absorbance. This can further be confirmed by the coefficient of determination value that is nearing 1 which is 0.9422. Coefficient of the determination value that is nearing 1 represents a strong relationship which suggests the model accurately representing the relationship between the concentration and absorbance in the tested range. However, higher absorbance value obtained for the sample introduces a potential inaccuracy due to extrapolation since it exceeds the range of the standard curve.

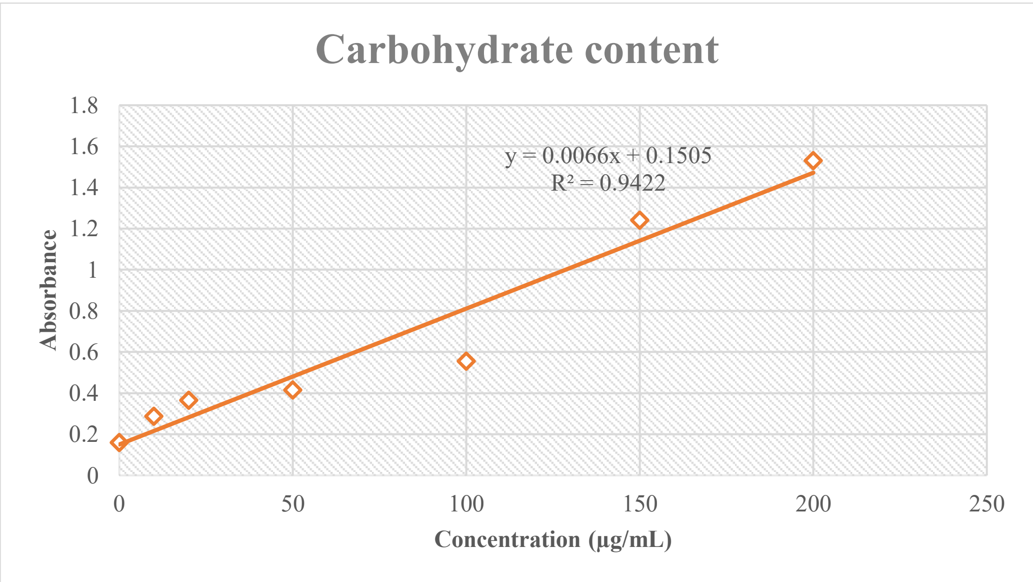

Figure 2. Carbohydrate content standard solution calibration curve.

Table 6. Sample carbohydrate content of absorbance and concentration.

Sample	Absorbance	Concentration (mg/L)
1	2.929	0.4210
2	3.237	0.4677
3	3.515	0.5098

At absorbance of 2.929, Sample 1 carbohydrate concentration is 0.4210 mg/ml which is the lowest carbohydrate concentration compared to all the samples due to highest percentage of meat (74%) and lowest percentage of mushroom (10%) meanwhile Sample 2 calculated carbohydrate concentration is 0.4677 mg/mL at 3.237 nm. with higher percentage of mushroom (20%) and lower percentage of meat (64%) compared to Sample 1. Sample 3 exhibits the highest concentration of carbohydrates (0.5098 mg/mL) at 3.515 nm. These trends emphasize that the incorporation of mushrooms in the meatball's mixture increases the content of carbohydrate since mushrooms are the primary source of carbohydrate. Particularly, the mushroom used in this experiment contains 6 g of carbohydrates per 100 g, including certain polysaccharides such as β-glucans, mannan, and chitin on a fresh weight [12] which contributes to the overall carbohydrate content of the meatball's formulation.

The carbohydrate contents increase directly proportionally as the mushroom content increases, which highlights the rich carbohydrate component in mushrooms. Sample 1 may align with some consumers' low-carbohydrate products but may lack the benefits contributed by the carbohydrate, such as texture improvement and moisture retention, while Sample 3 enhances texture and water holding capacity as it contains the highest carbohydrate content and Sample 2 poses as a balance, providing moderate carbohydrate content and giving better texture due to the inclusion of mushroom-derived polysaccharides [10].

Physical Analysis

Water Holding Capacity (WHC)

Increasing the mushroom percentage is supposed to significantly increase the water holding capacity (WHC) of the meatballs as the presence of primary polysaccharides that are present in large concentrations in the mushroom helps enhance the moisture retention and texture [13], thus making them a valuable ingredient in making sustainable meat reduced alternatives while also balancing the sensory attributes without compromising the quality for consumer satisfaction. Water holding capacity (WHC) is the capacity of muscle tissue to hold or lose water. The concept of water holding capacity is the capacity of native protein in the raw meat to bind water through the peptide bonds and hydrophilic lateral groups of protein macromolecules' amino acid residues to hold the opposite-charged ions and water dipoles. The terminal groups in the lengthy chains of fibrillar proteins, such as collagen, form chemical interactions with one another. Protein swelling is caused by the terminal groups' formation of a three-dimensional spatial structure, which holds and immobilizes water inside the spatial lattice. Globular proteins are made up of polypeptide chains that are rolled up so that the globe's surface has hydrophilic centers, and its interior has hydrophobic centers. Thus, when the meat is exposed to high heat, the denaturation and coagulation of the myofibrillar protein occur, which leads to the loss of water-holding capability [14]. By adding mushrooms in this meat concoction, it is supposedly increases the water-holding capacity of the meatballs since *Pleurotus sajor-caju* mushroom is rich in beta-glucans, which have hydrophilic characteristics that allow it to bind water efficiently. These characteristics are further enhanced due to the *Pleurotus sajor-caju* mushroom's sponge-like structure that absorbs water and the presence of specific polysaccharides, such as mannan, that can form gels which increase water retention in the meatball matrix [15].

This trend can be further confirmed by the results obtained (**Table 7**) and calculated based on Equation 3. Sample 1 contains 74% of meat and 10% of *Pleurotus sajor-caju* mushroom mixture shows the lowest WHC at 8.50% among all three samples. This is due to a higher meat proportion reducing the ability of the meatball to hold water after the processing and specifically, meat has the tendency to denature at high heat, which eventually releases bound water, thus leading to lower WHC. Sample 2 (64% meat and 20% mushroom) shows an increase in WHC to 12.5% compared to Sample 1, while Sample 3 exhibits the highest WHC at 15% compared to all three samples since Sample 3 contains a 54% meat and 30% mushroom mixture.

Table 7. Water holding capacity (WHC) percentage.

Sample	Weigh before (g)	Weigh after (g)	WHC (%)
1	2	1.83	8.5 ± 0.87
2	2	1.75	12.5 ± 0.54
3	2	1.70	15.0 ± 1.07

As the proportion of meat decreases and mushrooms increase, it improves the moisture retention of the meatballs due to increasement of beta-glucans presence which increases the hydrophilic nature of the meatballs [15]. This further improved the sensory properties such as the meatballs' juiciness and texture since Sample 3 can hold water more efficiently compared to Samples 1 and 2. These findings highlight mushroom-based meatballs' potential in addressing sustainable goals while also retaining the desirable texture and reducing reliance on animal-derived ingredients reliance.

CONCLUSION

In conclusion, the addition of *Pleurotus sajor-caju* into a meatball formulation shows strong potential as a strategy to reduce meat usage while maintaining desirable nutritional and qualities. Increasing mushroom content notably reduces fat content and improved water holding capacity and moisture. Thus, present promising alternative to traditional meat products.

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