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Physicochemical, Microbiological Characteristics of Meatballs from a Mixture of IPB D1 Chicken and Local Indonesian Rabbit Meats

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Abstract: IPB D1 chicken meat originates from a novel hybrid domestic chicken lineage, possessing nutritional attributes comparable to those found in rabbits. IPB D1 chicken and rabbit meat are rich in protein and minerals and can be used as the main ingredients for nutrient-rich food products, such as meatballs. This study aims to analyze the physicochemical, microbiological, and organoleptic characteristics of meatballs made with 100% chicken meat from IPB D1, 100% rabbit meat, and a combination of 50% chicken meat from IPB D1 and 50% rabbit meat. The meatballs were tested for their physical characteristics, such as pH, water activity, cooking loss, and texture profile, alongside chemical, microbiological, and sensory test characteristics to identify which formulation produced a meatball with optimal results. The study employed a completely randomized design. Results showed that meatballs with a composition of 50% IPB D1 chicken meat and 50% rabbit meat exhibited superior physical, chemical, microbiological, and sensory test outcomes. Results obtained from this study are promising for development into functional and diverse food products, as they have potential for commercial advancement.

Keywords: *IPB D1 chicken meatball, rabbit meatball, physicochemical*

1. Introduction

Many Indonesians still suffer from malnutrition and various diseases due to inadequate animal protein consumption. According to BPS, meat consumption in Indonesia remains relatively low and declined in March 2021. The Central Statistics Agency in 2021 stated that purebred or free-range chicken consumption was recorded at 0.538 kg/capita/month, which decreased by 3.4% from the previous year at 0.557 kg/capita/month, whereas rabbit meat consumption only reached 0.27 kg/capita/year. A lack of animal protein can cause diseases such as iron deficiency anemia and toddler stunting. Therefore, animal protein consumption is significant for the sustainability of community nutrition. This problem can be addressed through food diversification, a strategy aimed at reducing malnutrition risk by consuming various types of food with different nutritional content.

Food diversification can aid in preventing micronutrient deficiencies that cause health issues such as anemia and vitamin deficiency. Moreover, food diversification can enhance food security in an area by boosting local food production and lessening dependence on limited food sources (Setyawardani et al., 2020). This approach can be a solution to improve the nutritional quality of Indonesian people, who are still fixated on consuming imported broiler chicken and red meat.

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The IPB-D1 chicken is a new family of composite local chickens developed through a crossbreeding program between the F1 PS male (Pelung × Sentul) and the F1 KM female (Kampung × Cobb parent stock) (Habib et al., 2020). The establishment of IPB-D1 chickens aims to increase the participation of local chickens, which can utilize local feed, grow fast, and reach slaughter weight at 10-12 weeks. Their adaptability to the environment is quite good, with strong body resistance to New Castle Disease (ND) or Tetelo and Salmonella. IPB-D1 chicken meat is rich in protein content at 18.35% and minerals, especially Fe, Zn, Mn, and Se, which can prevent diseases such as anemia and stunting in children. Using IPB-D1 chicken can also increase protein diversification and reduce the consumption of imported broiler chickens, thus promoting food self-sufficiency and meeting food needs. A livestock commodity with good carcass potential, aside from chicken, is rabbit, which has high nutritional value and potential as a consumable meat for the Indonesian population.

Rabbit meat is less desirable than other animal meat. Rabbit meat exhibits a 20-21% protein content, with easily digestible amino acids and a relatively low fat content. Additionally, rabbit meat has a reasonably high mineral content, specifically Fe at 6 mg/kg and Zn at 13.23 mg/kg (Weller et al., 2014). Although using rabbit meat as a food ingredient is still relatively low and standard for the community, it can be improved through processing technology. One of the processed products that can compete in the market is meatballs. Considering that meatball products are trending among Indonesians, with the consumption rate reaching 31.4 portions per capita per year and increasing annually by 17.6% (Secretary General of Agriculture of the Republic of Indonesia,

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2018). The use of rabbit meat still needs to be promoted, so innovation efforts are underway to combine chicken and rabbit meatballs to increase public consumption of rabbit meat in Indonesia. With the high nutritional value of IPB-D1 chickens and local rabbits, product diversification can be developed, enhancing food quality, preventing disease, and curtailing the increase of import trade by prioritizing local meat. This can be developed in underdeveloped villages to stimulate the regional economy.

Meatballs combining IPB-D1 chicken and rabbit, rich in protein and minerals, can mitigate the risk of stunting and anemia. Consequently, further research is necessary to assess the physicochemical, microbiological, and organoleptic qualities of meatballs incorporating IPB-D1 chicken and rabbit meat.

2. Materials and Methods

Time and Location

This research was conducted from June to August 2022 at the Integrated Laboratory of the Department of Animal Production Science and Technology, Faculty of Animal Science, IPB University, Indonesia.

Materials

The tools used were a meat pH meter, biuret, pipette, freezer (-18°C), petri dish, hot plate stirrer, test tube, Erlenmeyer tube, digital scale, measuring cup, micropipette, Bunsen heater, incubators, refrigerators, autoclaves, vortexes. Tools in the organoleptic test include paper plates, knives, forks, questionnaire paper, and stationery. The raw meatball materials utilized were IPB D1 chicken, rabbit meat, salt, pepper, tapioca flour, ice cubes, garlic powder, and Sodium tripolyphosphate (STPP).

Methods

Preparation of meatballs

The procedure for meatball production referred to Kia et al. (2016). Five hundred grams of cleaned and deboned meat was cut into small pieces and then ground using a food processor. The seasonings containing 3% salt, 0.3% sodium tripolyphosphate, 10% tapioca, 35% ice, 0.5% pepper, 0.3% garlic powder, and other ingredients were added to the food processor and mixed thoroughly. The dough was molded into rounds and placed in hot water at 80°C for 7–10 minutes. After that, each meatball sample was separated according to the needs of the analysis.

Physical Analysis

The physical analysis includes pH, cooking loss, water absorption, and texture profile. The pH meter was first calibrated with buffer solutions at pH 4 and 7 according to SNI (1995) guidelines. The electrodes were rinsed with distilled water and dried, and the pH meter was inserted into the meat sample approximately 2-4 cm. The pH value was obtained by reading the scale. Cooking loss is calculated according to Bouton (1971), where cooking loss is measured as the difference in the amount of water after centrifugation and the initial amount of water before boiling. The value obtained was calculated using the formula from Fardiaz (1992). The texture profile of the meatball was measured using a Texture Analyzer Perten Instrument TVT6700 with a cylindrical probe of 20 mm, a compression rate of 80%, and a probe speed of 1 mm/s.

Chemical Analysis

Chemical analysis (water content, ash content, protein content, fat content, and carbohydrate content) was performed using the modified AOAC (2005) method. Mineral analysis employed the wet ashing method by Reitz (1960). Cholesterol analysis used the Chromatography Flame Ionization Detector according to the ASEAN Manual of Nutrient Analysis (2011) method.

Microbiological Analysis

Total Plate Count Analysis or total microbes was measured by the BAM (2001) method using Plate Count Agar as the medium, and the dilutions used were 10-2, 10-3, and 10-4 for meatball products. The calculation was performed using the pour plate method when visible colonies were at 25-250. Total Escherichia coli analysis was performed by the BAM (2001) method using Eosin Methylene Blue Agar (EMBA) media, and the dilutions used were 10-1, 10-2, and 10-3 for meatball products.

Sensory Test

Sensory quality was assessed by 40 semi-trained panelists based on the modified Setyaningsih (2010) method. The method used for the sensory test was the hedonic test (preference test) and the hedonic quality test, which evaluated the meatballs' color, aroma, taste, texture, and general appearance. The hedonic and hedonic quality tests used a scale of 1 to 5. The hedonic quality scale was applied to color, aroma, taste, and texture.

Data Analysis Method

The experiment was conducted in a completely randomized design with three treatments, namely P1 (100% chicken meat from IPB D1), P2 (100% rabbit meat), and P3 (a combination of 50% chicken meat from IPB D1 and 50% rabbit meat). Each treatment was replicated four times, and the obtained data were subjected to an analysis of variance (ANOVA) in a Randomized Block Design, followed by Tukey's test to determine significant differences.

3. Result and Discussion

Meatballs Physical Characteristic

The results of the physical properties (pH, water absorption, cooking loss, and profile texture) are shown in Table 1. The pH value showed a significant difference at varying meatball formulations.

Table 1. Result of physical characteristic test

Parameter	Treatments		
	P1	P2	P3
pH	6.03±0.01[a]	6.20±0.02[c]	6.10±0.01[b]
Cooking Loss (%)	2.21±0.02[b]	2.55±0.14[c]	1.79±0.09[a]
WA (%)	4.5±0.41[a]	4.25±0.35[a]	5.25±0.25[b]
Profile Texture *Cohesiveness*	0.19±0.03[a]	0.19±0.01[a]	0.20±0.02[b]
Springiness	0.70±0.03[a]	0.78 ±0.15[b]	0.84±0.05[c]

(P1; 100% IPB D1 chicken meat, P2; 100% rabbit meat, P3; 50% IPB D1 chicken meat+50% rabbit meat, F4)

[a] means with different superscript are significantly different (P<0.05)

The highest pH was found in P2, with a value of 6.20. Widati et al. (2022) state that the pH of rabbit meatballs ranges from 6.59 to 6.64, indicating that the pH value is consistent with this study. According to Islam et al. (2018), the pH of chicken meatballs is 6.04. The pH can be affected by glycogen levels in different types of meat, thereby influencing the rate of glycolysis. Muscle glycogen levels impact the production of lactic acid and the pH of the meat. Low glycogen levels result in a high pH and slow down glycolysis. In addition, to decrease the pH, glycogen converts into lactic acid in the meat post-slaughter due to an extensive biochemical process. This causes acidification in the meat, leading to a decrease in the pH value. The high pH is also influenced by dietary fiber, which affects meatball pH. Several factors, such as muscle metabolism type and fiber, influence glycogen concentration, impacting the pH of meatballs. The cooking process can increase the pH value of meatballs because cooking can release bonds containing sulfhydryl, imidazole, and hydroxyl groups in the meat (Uzun and Oz., 2021).

Measurements of cooking losses showed significant differences in meatball samples with varying types of meat composition. The range of cooking losses in this study was 1.79-2.55%, with the lowest value being the combination treatment of rabbit meat and chicken IPB D1, at 1.79%. Cooking loss is an assessment of the nutritional value of meat related to its water content. During cooking, meat loses volume and weight due to the release of fluids; water is lost and melted fat drips from the product due to protein denaturation caused by heat (Mena et al., 2019). The decrease in the percentage of cooking loss in the meatballs could be due to the addition of specific types of meat, which resulted in reduced cooking loss; this could be attributed to the combination of a mixture of IPB D1 rabbit meat and chicken, which may increase the protein content more than other treatments. Jia et al. (2020) stated that protein could bind water, forming more hydrogen bonds, and high protein content will increase water-holding capacity, which can affect cooking shrinkage. According to research by Frunza et al. (2023), the protein content of rabbit meat in the Longissimus dorsi section is 21.6%, and the protein content of IPB D1 chicken is 17-18%. Water absorption capacity is inversely proportional to cooking losses, so the higher the water-holding capacity, the lower the cooking losses.

Furthermore, the higher the cooking losses, the more nutrients will be lost from the meatballs during the cooking process. Meatballs of good quality have a lower cooking loss value (Patriani and Apsari, 2022; Malini et al., 2016). This is consistent with the results of the highest protein content in P3, which showed the lowest cooking loss value. The measurement of water absorption capacity showed a significant difference between the samples. The range of water absorption observed was 4.25-5.25%, with the highest yield obtained by P3.

In contrast, the lowest result was obtained by P2. Apriantini et al. (2021) stated that a high water absorption capacity in meat products results in minimal water loss, leading to a better meat structure. Increased water absorption enhances the texture value. Therefore, the highest value in P3 is the meat combination of chicken and rabbit. The decrease in water absorption is likely due to the denaturation of some proteins. This is supported by Hutabarat et al. (2021), who stated that water absorption affects the cooking shrinkage value. The cooking process causes changes in water absorption due to protein solubility in meat. High temperatures increase protein denaturation and reduce water absorption. All parameters of the physicochemical qualities of meatballs fitted the quality of animal products such as sausage and yogurt (Sulaiman et al., 2016; Astawan et al., 2012).

Furthermore, the higher the cooking losses, the more nutrients will leach from the meatballs during the cooking process. Meatballs of good quality have a lower cooking loss value (Patriani and Apsari, 2022). This is consistent with the results demonstrating that the highest protein content in P3 corresponds to the lowest cooking loss value.

Measurement of water absorption capacity demonstrated a significant difference between the samples. The range of water absorption obtained was 4.25-5.25%, with the highest yield found in P3. In contrast, the lowest result was obtained by P2. Apriantini et al. (2021) stated that high water absorption capacity of meat products results in minimal water loss, leading to better meat structure. Increased water absorption enhances the texture value. Therefore, the highest value in P3 reflects a meat combination of chicken and rabbit. The decrease in water absorption is likely due to the denaturation of some proteins. This is reinforced by Hutabarat et al. (2021), who stated that water absorption influences the cooking shrinkage value. The cooking process causes changes in water absorption due to the protein solubility in meat. High temperatures increase protein denaturation and reduce water absorption. All parameters of physicochemical qualities of meatballs fit the quality seen in animal products such as sausage and yoghurt (Sulaiman et al., 2016; Astawan et al., 2012).

3.1 Input Point Cloud Datasets

This study uses three different datasets for experimental verification purposes: (i) box, (ii) cup, and (iii) Stanford bunny obtained from the PointCleanNet database.

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Meatballs Chemical Analysis

Based on the results of the research, chemical analysis such as fat, protein, carbohydrate, mineral and cholesterol that was significantly different.

Table 2. Chemical Analysis test results

Parameter	Treatments		
	P1	P2	P3
Water Content (%)	73.92±0.08	72.51±0.01	74.38±0.05
Ash (%)	2.68±0.01	2.34±0.07	2.35±0.02
Fat (%)	1.13±0.06[b]	2.34±0.03[c]	1.06±0.02[a]
Protein (%)	15.41±0.05[a]	15.17±0.35[a]	16.71±0.20[b]
Carbohydrate (%)	6.86±0.08[b]	7.65±0.43[c]	5.50±0.26[a]
Mineral			
Fe (ppm)	12.25±0.10[a]	13.15±0.13[b]	15.76±0.10[c]
Zn (ppm)	7.36±0.02[a]	8.23±0.02[b]	10.15±0.02[c]
Mn (ppm)	0.23±0.03[a]	0.39±0.03[b]	0.46±0.03[c]
Cholestrol (mg/100g)	33.33±0.01[a]	37.55±0.16[c]	36.30±0.13[b]

(P1; 100% IPB D1 chicken meat, P2; 100% rabbit meat, P3; 50% IPB D1 chicken meat+50% rabbit meat)

[a] means with different superscript are significantly different ($P<0.05$)

The water content in meatballs made from three different types of meat showed no significant difference. The highest water content was obtained in P3, with 74.38%. According to the Indonesian National Standard, the maximum water content is 70%, meaning that all three treatments did not meet the standard. The water content of food ingredients determines the acceptability and durability of these materials. Most changes in food ingredients occur in water media added or derived from the ingredients themselves (Persson et al., 2003). The heating time and the fillers affect the water content in the meatballs. Prolonged heating increases water absorption because water can diffuse into the food and bind starch and protein (Pramuditya & Yuwono, 2014).

The ash content of the meatballs in this study ranged from 2.34% to 2.68% and showed no significant differences between treatments. According to SNI, the maximum ash content in meatballs is 3%. Salazar et al. (2018) stated that the ash content of rabbit meatballs soaked in tamarillo juice was 1.16% to 2.66%. Thus, the ash content of the meatballs in this study did not exceed the SNI limits.

The average fat content of the meatballs using three different treatments ranged from 1.06% to 2.34% and showed a significant difference between the meatballs (Table 2). The combination of IPB D1 rabbit and chicken meatballs had the lowest fat content (1.06%). This is presumably due to the relatively low-fat content of IPB-D1 chicken meat. The average fat content of IPB-D1 chicken breast ranged from 0.15% to 0.32%. Nistor et al. (2013) stated that the fat content of rabbit meat was around 9.2 g/100 g sample. According to SNI, meatball products have a maximum fat content value of 10%. Thus, the fat content of meatball products in this study is still within the safe range of the Indonesian National Standard. The fat content of meat is closely related to its water content; the greater the fat content, the lower the water content (Berutu et al., 2010). This is consistent with the results of this study, where the water content of the three treatments has a negative correlation with the fat content value.

The protein content of the meatballs with the three different meat compositions ranged from 15.27% to 16.71%, indicating that the protein content of the meatballs had a significant difference (Table 2). According to SNI 01-3818-1995, the minimum protein content of meatballs is 11%. Thus, the protein content of P1 and P2 fulfills the SNI requirements. Meanwhile, according to SNI, the minimum protein content of combined meatballs is 8%, indicating that P3 has fulfilled the SNI requirements. The highest protein content was found in meatballs P3, with 16.71%. This can be influenced by the high or low protein content of the raw meat used in the dough. Rabbit meat has a protein content of 20-21% (Sutaryo et al., 2021). Nusi (2010) states that high and low levels of meat protein are related to water and fat content. Protein content will increase if the intramuscular fat content is low and the water content is high. Protein content was effected by physical treatment and proteolytic enzyme during processing (Afiyah et al., 2015). The longer the boiling, the more proteins are damaged, and boiling at high temperatures will cause denaturation, causing the protein content to drop.

The carbohydrate composition of the raw materials impacted the carbohydrate content of the meatball samples. Carbohydrate levels in IPB D1 chicken meatballs, rabbit meat, and their combinations showed significant differences. The value of carbohydrate content in this study was 5.50% to 7.65%. Meatballs with 100% rabbit meat treatment have the highest carbohydrate content value. Water and protein content are related to the carbohydrate content of meatballs; the higher the water content and protein content, the lower the carbohydrate content of meatballs (Rahmah and Nurul, 2021). The average carbohydrate value of IPB-D1 chicken breast meat in this study ranged from 3.81% to 5.45%. According to Grace and Henry (2020), ingredients containing carbohydrates are present in tapioca flour, which has 89.04%.

The mineral content of the meatballs shown in Table 2 reveals a significant difference. Meatball Fe values in this study ranged from 12.25 ppm to 15.76 ppm. The Zn content was 7.36 ppm to 10.15 ppm, and the Mn level was 0.23 ppm to 0.46 ppm. P3 obtained the highest Fe, Zn, and Mn values. This is due to the high Fe mineral content in IPB D1 chicken meat, 16 mg/kg, and the mineral content in rabbit meat, 6 mg/kg Fe and 13.23 mg/kg Zn (Weller et al., 2014). Martinez et al. (2018) reported the content of control chicken meat without treatment had an Fe content of 10.65 mg/kg and Zn of 1.49 mg/kg. The high mineral content of IPB D1 chicken can be considered a functional food that can be beneficial for sufferers of iron diversification anemia and stunting in children.

The meatball cholesterol levels for the three treatments are presented in Table 2. The results showed that the highest cholesterol level was found in P2 (100% rabbit meat) with a score

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of 37.55 mg/100g, while the lowest score was recorded by P1 (chicken meatballs with 100% IPB D1) of 33.33 mg/100g. It is suspected that IPB D1 chicken meat has low cholesterol content. The cholesterol content of rabbit meat in the study by Puspani et al. (2019) of 48.07 mg/dg also explained that a decrease in the activity of lipogenic enzymes and an increase in the excretion of bile acids in the feces partly caused the reduction in cholesterol.

Meatballs Microbiological Analysis

Measurement of microbiolgical analysis was performed to detect the presence of microorganisms in a food product. The results of the three different treatments of meatballs are presented in Table 3 below.

Table 3. Microbiological Analysis test results

Parameter	Treatments		
	P1	P2	P3
Total Microbes (log cfu/g)	3.01±0.24^{b}	3.03±0.13^{b}	2.68±0.13^{a}
Total *E. coli*	Negative	Negative	Negative

(P1; 100% IPB D1 chicken meat, P2; 100% rabbit meat, P3; 50% IPB D1 chicken meat+50% rabbit meat)

^{a} means with different superscript are significantly different (P<0.05)

The results of microbial analysis for the three treatments showed a significant difference. Using rabbit meat in meatball products resulted in the highest number of microbes, namely 3.03 log cfu/g. In comparison, meatballs with a combination of IPB D1 chicken and rabbit meat had the lowest microbial count, at 2.68 log cfu/g. According to SNI 01-3818-2014 regarding microbial contamination, the allowable plate numbers for meatball products and combined meatballs are a maximum of 1 x 10^{5} colonies/g (5.0 log cfu/g). Therefore, the three meatball treatments met the quality requirements for total microbial content. *Escherichia coli* is a foodborne pathogen that causes diarrhea in humans, especially children and the elderly, and is characterized by acute kidney failure, hemolytic anemia, and thrombocytopenia (Sallam et al., 2021). Numerous materials are used as preservative agents in animal products, such as bacteriocin (Hanifah et al., 2016; Sihombing et al., 2015), teak leaf extract (Arief et al., 2014) and lactic acid bacteria which have antimicrobial properties (Adiyoga et al., 2022). Quantitative data from the study demonstrated that *Escherichia coli* levels in this study were negative (Table 3). The three meatball treatments met the requirements for being free from *E. coli* contamination. This is due to the entire process of making meatballs being conducted hygienically.

Sensory Test

Sensory tests include hedonic and hedonic quality tests. The color, taste, texture, and general appearance in the hedonic test showed that the results were significant. The average value of the hedonic quality test for meatball texture demonstrated a significant difference.

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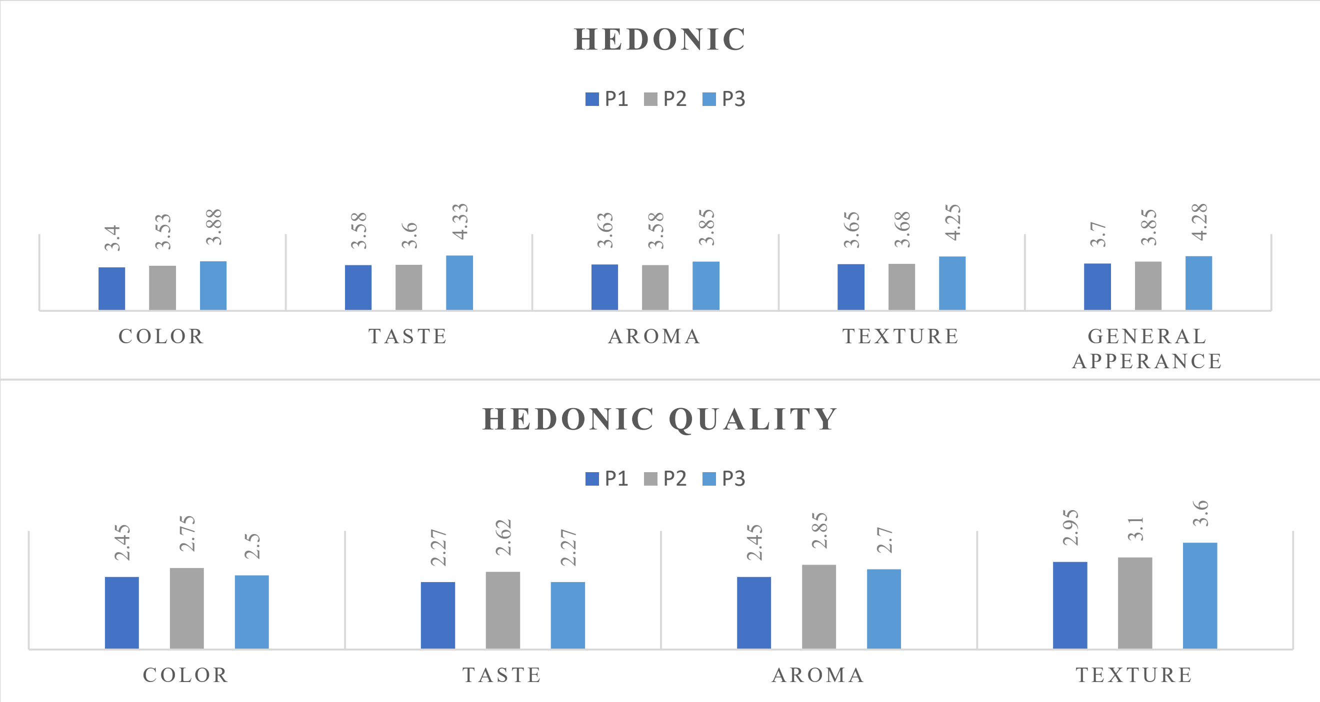

Figure 1. Sensory Test. P1 = 100% chicken meat from IPB D1, P2 = 100% rabbit meat, P3 50% chicken meat from IPB D1 + 50% rabbit meat. Hedonic scale 1 (dislike very much), 2 (dislike), 3 (kinda like or quite like), 4 (like), 5 (like very much). Color hedonic quality: scale 1 (very white), 2 (white), 3 (slightly gray), 4 (gray), 5 (very gray). Flavor: 1 (very meaty), 2 (meaty), 3 (slightly meaty), 4 (no meaty taste), 5 (very no meaty taste). Aroma: 1 (very meaty), 2 (meat-scented), 3 (slightly meaty), 4 (unscented meat), 5 (very unflavored meat). Texture: 1 (very not chewy), 2 (not chewy), 3 (slightly chewy), 4 (chewy), 5 (very chewy).

The panelists preferred the taste, texture, and general appearance of P3 meatballs (comprising 50% rejected IPB D1 chicken meat and 50% rabbit meat), compared to the other two treatments. Panelists showed quite favorable results for meatballs in the three different treatments regarding aroma and color. The release of cell fluids during cooking will reduce the intensity of the color along with the release of pigments. The panelists preferred the taste of meatballs with a mixture of IPB D1 chicken meat and rabbit meat (P3) compared to other meat compositions.

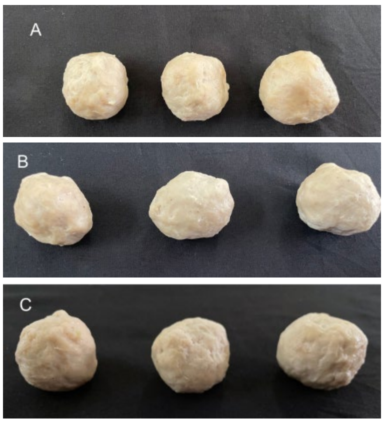

Figure 2. Sensory Test. A = 100% chicken meat from IPB D1, B = 100% rabbit meat, C= 50% chicken meat from IPB D1 + 50% rabbit meat.

The score obtained for the "taste" of IPB D1 chicken and rabbit combination meatballs reached a value of 4.33 (the highest score), indicating that the IPB D1 chicken and rabbit combination meatballs were acceptable to consumers. The formulation between meat and the primary raw material, combined with flour and other supporting ingredients, significantly affects the taste of the meatballs, and this is reinforced by Gedrovica and Karklina et al. (2013). The added formulation between meat and raw materials will significantly influence the taste of the meatballs because it will impact the taste of the product, and this means that sensory evaluation of products is critical, especially for new products like this one. The mean values of the hedonic quality test for color, aroma, and taste also showed insignificant differences. In the color assessment, the three treatments showed that the meatballs were white. In the aroma assessment, the three meatballs could be interpreted as smelling of meat, while in the taste assessment, the three treatments had the result that the meatballs had a distinctive taste of meat. The texture value of the meatballs showed a significant difference; P3 had a slightly chewier texture than other treatments. High connective tissue content in meat can cause the rough texture of processed products. High connective tissue makes it difficult for the meat to be ground smoothly, resulting in a less chewy or rough texture.

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4. Conclusion

The use of 50% IPB D1 chicken meat and 50% rabbit meat for meatball composition showed more effective results than the other treatments based on parameters of physical characteristics, chemical, microbiological, and sensory tests. Meatballs mixed with IPB D1 chicken and rabbit meat can be used for food diversification and as a food approach in society.

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