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Physicochemical evaluation of chicken liver enriched sausages for maternal nutrition and stunting prevention

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ABSTRACT

Stunting is a condition of chronic malnutrition resulting from prolonged inadequate nutritional intake, particularly during early childhood. A contributing factor is maternal malnutrition during pregnancy, including chronic energy deficiency and anemia. Therefore, this study aimed to formulate and analyze the physicochemical, microbiological, and organoleptic characteristics of chicken meat sausages enriched with chicken liver, kidney beans, skim milk, and carrageenan. These ingredients were selected for their high nutritional value, particularly heme iron, which is more bioavailable and may contribute to reducing stunting risk. In addition, the organoleptic test results of sausages showed a significant difference in the sensory test of sausages with a p-value <0.05. The optimal formulation was identified as treatment A3K1 (chicken liver 32.5: kidney beans 17.5: skim milk 15: carrageenan 1). The proximate analysis for formula A3K1 included ash content of 3.11%, moisture 55.31%, calories 199.35 kcal, protein 18.81%, carbohydrates 16.16%, fat 6.61%, iron 9.27%, and calcium 45.25%. In the chemical test, significant results were obtained with a p-value < 0.05. The chewiness ranged from 722.57 to 1175.32 gf, water holding capacity (WHC) between 1.41 and 2.37%, and cooking loss from 4.69 to 4.75%. While had a significant effect with a p-value <0.05. Microbiological analysis confirmed the absence of *Escherichia coli* and *Salmonella spp* among all samples. The inclusion of chicken liver, kidney beans, skim milk, and carrageenan significantly enhanced the physicochemical properties and nutritional profile of the sausages, indicating its potential to support maternal nutrition and contribute to stunting prevention strategies.

Contribution/Originality: This study originally used chicken liver to develop sausages as a functional food for maternal health. This research combined the benefits of chicken liver, kidney beans, skimmed milk, and carrageenan to increase the nutritional value of chicken sausages as an effort to prevent stunting. The nutritional information of chicken liver sausages includes physicochemical analysis, organoleptic evaluation, and safety quality measurement.

1. INTRODUCTION

Stunting is a condition of poor nutritional status caused by a mismatch between intake and nutritional needs over a long period, particularly during the First 1000 Days of Life or when the fetus is two years old (Handayani, 2023). The prevalence of stunting according to WHO in 2022 has decreased by 22.3%, but progress has been slow over the last 5 years (WHO, 2023). Meanwhile, in Indonesia, according to the Ministry of Health's Indonesian Nutrition Status report, in 2022, it was 21.6%, with the majority of children aged 3-4 years at 6% (Munira, 2023). This incident has causative factors such as anemia conditions that occur during the first trimester and can cause poor fetal development and a decrease in essential nutrients. Achieving optimal nutritional status must be accompanied by adequate nutrition during pregnancy, as recommended in the prevention of stunting (Smith, Johnson, & Brown, 2023) including nutrients such as protein, energy, iron, zinc, calcium, folic acid, and omega 3 fatty acids and other essential types (Christian et al., 2013; Saleh, Syahrul, Hadju, Andriani, & Restika, 2021). So, one solution to increase iron consumption through functional food is to create innovative foods based on local food ingredients for pregnant women in the form of sausages, adding chicken liver, red beans, skimmed milk, and carrageenan.

Chicken liver is a source of animal protein that contains vitamins and minerals such as vitamin A, vitamin B12, folic acid, zinc, and iron, and is a source of heme iron, which has a higher absorption value in the body (Xiong, Gao, & Zheng, 2017). Processing chicken liver affects the nutritional value in it and affects hedonics or sensory when consumed (Qu et al., 2021). So, in some studies, the processing of chicken liver varies, such as in the form of flour for longer storage. The addition of various concentrations of chicken liver flour has a significant effect on protein, iron, and calorie levels of processed spice cookies (Pratiwi, Lestari, Yulistiani, & Rosida, 2023). This is in accordance with a similar food innovation, namely the making of chicken liver sausage as a prevention of anemia in adolescent girls, with the best nutritional value found in formula 4 (Chicken liver 55%: Soybeans 45%), producing 17.21 g protein and 7.415 mg Fe (100 g) (Lutfiah, Adi, & Atmaka, 2021). There are other similar studies on the prevention of anemia. Formula F1 (chicken liver 35%: tuna fish 65%) produced an analysis of energy content of 155 kcal, 12.5 g protein, 2.6 g total fat, 20.4 g total carbohydrates, 62.6% water content, 1.9% ash content, and 3.5 mg iron content (Rahmawati, Umami, Komalasari, Rinaldy, & Iskandar, 2025). The novelty of this research is supported by the addition of other ingredients such as kidney beans and skim milk to increase the nutritional value of chicken liver sausage, aiming to prevent and reduce the incidence of anemia in pregnant women, which will affect the increase in stunting in the future.

Consumption of kidney beans can also support the development of blood cells in the foetus (Bakara, Kamalah, Situmorang, & Sorong, 2022). Red beans for pregnant women with anemia or lack of nutrition affect the increase in hemoglobin and calories to meet the needs of pregnant women, especially in the third trimester. This is in accordance with the research of (Lamana et al., 2024) that a dose of 50g per day combined with Fe tablets for 14 days can increase hemoglobin levels in pregnant women. Skim milk plays a role in the formation of bones and teeth, so it can help optimal bone growth in the fetus. Carrageenan can improve organoleptic quality as a natural thickener and is useful in lowering blood lipid levels, improving the digestive system, and as an antioxidant because it contains vitamin C (Chodijah, Dewi, Jauhari, & Aji Kurniawan, 2022).

Therefore, this study aimed to formulate and analyze the physicochemical, microbiological, and organoleptic characteristics of chicken meat sausages enriched with chicken liver, kidney beans, skim milk, and carrageenan, including ash content, moisture content, calories, protein, carbohydrates, fat, iron, calcium, chewiness, water holding capacity (WHC), cooking loss, and testing for E. coli and Salmonella spp. Sausages produced are useful for improving the nutrition of pregnant women to prevent stunting.

2. MATERIAL AND METHOD

2.1. Materials and Equipment

The ingredients used in this research include chicken meat, additional ingredients (chicken liver, red bean flour, skimmed milk), a chewing agent (carrageenan), seasonings, colourings, other additional ingredients, and sausage casings. Meanwhile, the equipment used in this study included a chopper (dough grinder), spoon, digital scale, grinder, 80-mesh sieve, oven, steamer, knife, piping bag, syringe, string, and tongs. All reagents and chemical solutions were of analytical grade. The specific formulations used in this study are detailed in Table 1, which presents the percentage of four main ingredients used across twelve experimental groups: three control groups (A, B, and C) and nine treatment groups (D through L). The formulation includes chicken liver, red bean flour, skimmed milk, and carrageenan. The control groups (A0K1, A0K2, A0K3) do not contain chicken liver, red bean flour, or skimmed milk, but they vary in carrageenan percentage (1%, 1.25%, and 1.5%). Conversely, the treatment groups are categorized based on three levels of chicken liver weight percentage (A1: 22.5%; A2: 27.5%; A3: 32.5%), each combined with varying percentages of carrageenan (K1: 1%; K2: 1.25%; K3: 1.5%), with corresponding adjustments to red bean flour and skimmed milk content.

Table 1. Chicken sausage formulation.

Formulation	Units	Control			Treatment								
		A0K1 (A)	A0K2 (B)	A0K3 (C)	A1K1 (D)	A1K2 (E)	A1K3 (F)	A2K1 (G)	A2K2 (H)	A2K3 (I)	A3K1 (J)	A3K2 (K)	A3K3 (L)
Chicken Liver	%	-	-	-	22.5	22.5	22.5	27.5	27.5	27.5	32.5	32.5	32.5
Red Bean Flour	%	-	-	-	27.5	27.5	27.5	22.5	22.5	22.5	17.5	17.5	17.5
Skimmed Milk	%	-	-	-	10	10	10	12.5	12.5	12.5	15	15	15
Carrageenan	%	1	1.25	1.5	1	1.25	1.5	1	1.25	1.5	1	1.25	1.5

Note: A0; A1; A2; A3: chicken liver weight percentage, K1; K2; K3: Carrageenan percentage.

2.2. Preparation and Formula Chicken Sausages

The formulation of chicken sausages with additives and chewers used a completely randomized design (CRD) with 2 factors and a 3x3 factorial arrangement. Variations of chicken liver, kidney beans, and skimmed milk formulation (A) to be used include 3 levels, namely 45g: 55g: 20g (A1), 55g: 45g: 25g (A2), 65g: 35g: 30g (A3). The formulation of carrageenan addition (K) is 10g (K1), 12.5g (K2), and 15g (K3). The treatment variations are as follows:

The sausage-making procedure involves modified recipes based on various studies (Hastuti, Rahmawati, Muharexza, & Choironi, 2023; Ismanto, Lestyanto, Haris, & Erwanto, 2020). Chicken meat and chicken liver are ground using a chopper, then the prepared spices are added. Once homogeneous, red bean flour, cornflour, skimmed milk, carrageenan, and ice cubes are incorporated, and the mixture is ground again. Subsequently, egg white and oil are added, and the mixture is ground until homogeneous. The sausage dough is then placed into collagen casings using piping bags and syringes. The sausages are steamed at low heat for 60 minutes. Finally, the sausage casings are removed.

2.3. Physicochemical and Organoleptic Tests of Sausages

2.3.1. Ash Content

Fresh sample 5g put into a porcelain cup that has been known weight, charred on a Bunsen with a small flame until smoky, then in the furnace at a temperature of 500-600^{0}C. The ash obtained was cooled in a desiccator and weighed to a fixed weight.

2.3.2. Moisture Content

5 g sample was weighed and then placed into a porcelain cup of known weight, which was subsequently put in an oven at 100-105^{0}C for 24 hours until the weight was constant. The cup containing the sample was removed from the oven and cooled in a desiccator.

2.3.3. Calorie (Calorimeter)

Determination of calorific value in sausages using the calorimeter method involves two stages: the first stage is determining the calorific capacity of the bomb calorimeter, and the second stage is measuring the heat of combustion of the substance. The process includes weighing approximately 1 g of the substance.

2.3.4. Carbohydrate Content (Luff Schoorl) (Ningrum, Angraini, Rahmawati, & Masruhim, 2024)

The principle is based on the ability of reducing sugars to reduce Cu (II) ions in alkaline Luff–Schoorl reagent to Cu (I) during boiling. The remaining unreduced Cu (II) is then quantified by iodometric titration: Cu (II) oxidizes iodide to iodine, which is titrated with standardized sodium thiosulfate solution. For total carbohydrates, the sample is first hydrolyzed with acid (inversion), converting non-reducing sugars into reducing sugars, then analyzed as above.

The difference between the blank titration and the sample titration corresponds to the amount of reducing sugars present. Results are expressed as glucose equivalents (or sometimes sucrose equivalents) in g/100 g or g/100 mL of sample.

2.3.5. Protein and Fat Content (SNI 01-2354.4-2006)

Samples (2 g) were digested with 15 mL H_2SO_4 and 3 mL H_2O_2, then deconstructed at 410°C for 2 hours. After cooling, 50 mL distilled water was added. The mixture was distilled with 4% H_3BO_3 and sodium hydroxide. Distillate was collected up to 150 mL. Finally, the distillate was titrated with 0.2 N HCl until the color changed from green to neutral grey.

2.3.6. Iron Content (AAS)

The sample (5 g) is diluted with 100 ml of distilled water and transferred to a 100 ml Erlenmeyer flask. The mixture is stirred evenly, and then 50 ml of the sample solution is taken. Subsequently, 5 ml of concentrated HNO_3 is added, and the flask is covered with a funnel. The mixture is heated slowly until the volume reduces to approximately 15-20 ml. The solution is then filtered using Whatman filter paper no. 42. After filtration, the filtrate (50 ml) is transferred to a glass container and made up to volume until homogenized. The spectrophotometer is then prepared for analysis, and the sample is analyzed accordingly.

2.3.7. Calcium Content (AAS)

The sample (10 g) was then heated on a hot plate at 120 °C for 2 hours. It was subsequently cooled in a desiccator for 24 hours, mixed with 2 ml of HNO_3, and dried on a heating plate. During the high deconstruction process, the sample was burned under closed conditions in a furnace at 500 °C for 2 hours to form carbon-free ash. After cooling at room temperature, the ash was dissolved with 1 ml of distilled water, and 10 ml of 2% HNO_3 was added. The solution was then filtered, and as much as 50 ml was dissolved with distilled water. A standard solution was prepared with $CaCO_3$ base by dissolving 250 mg in a solvent mixture of 5 ml HCl and 20 ml distilled water.

2.3.8. Water Holding Capacity (WHC)

Tests the ability of meat to bind water added from outside. To determine how meat maintains its water content when subjected to treatments such as cutting, heating, grinding, and processing. Add 10 mL of distilled water to 1 g of sample. Then, stir the suspension for 5 minutes and put the suspension into a centrifuge tube. Centrifuge the sample for 30 minutes at 3500 rpm. Separate the supernatant and weigh the sample precipitate.

2.3.9. Chewiness

Chewiness can be analyzed with Brookfield's Texture Analyzer CT3 (Dethan, Sabtu, & Riwu, 2022; Kusnadi, Bintoro, & Al, 2012). The elasticity of the sausages was analyzed using a trigger of 5.0 g, deformation of 10.0 mm, and a speed of 3.0 mm/s. Install the probe tool according to the material being tested. The probe used is a cylindrical probe with a diameter of 1 cm. The tested material is positioned directly at the bottom of the probe.

2.3.10. Cooking Loss

Bring 150 ml of water to a boil, then add 10 g of sausages and boil for 10 minutes. Remove and drain for 5 minutes. Collect the remaining cooking water and water from draining, then place in the oven until the weight is constant.

2.3.11. Escherichia Coli and Salmonella Test

The Escherichia coli test uses the Most Probable Number (MPN) method against Escherichia coli bacteria. Based on SNI 01-2332.1 - 2006, the MPN method consists of three stages (Katon, Solichin, & Jati, 2020). Meanwhile, the Salmonella sp. test uses 35 g of Atomic Absorption Spectrophotometry (AAS) media and 37.5 g of EMBA, each dissolved in 1 liter of distilled water, then brought to a boil and sterilized using an autoclave for 30 minutes at 121^{0}C, 1 atm (Fatiqin, Novita, & Apriani, 2019).

2.3.12. Organoleptic Evaluation

The organoleptic evaluation uses human senses to assess the level of preference for chicken liver sausages. Testing was carried out using organoleptic test form instruments with scoring/scaling tests and the allergy form. This was conducted with ethical approval from the Medical Research, Faculty of Dentistry, Universitas Jember, granted approval for this study on 19 November 2024 (Ref. No. 2848/UN25.8/KEPK/DL/2024).

2.3.13. Data Analysis

Data analysis used ANOVA parametric test to see significant differences in proximate and microbiological data, followed by Post Hoc Tukey test, while the protein test used Welch ANOVA and Games-Howell tests with a significant p-value <0.05, indicating a difference between the two values. Meanwhile, to measure and observe significant differences in organoleptic data, a non-parametric test was used, namely the Friedman test with Post Hoc test.

3. RESULTS AND DISCUSSION

3.1. Organoleptic Evaluation

Sausages with the addition of chicken liver are products without preservatives and synthetic additives that can be used as functional foods to increase adequacy and meet the consumption needs of macro- and micronutrients for pregnant women.

Table 2 presents the organoleptic characteristics of Chicken Liver Sausages, detailing the scores assigned to three control groups (A, B, and C) and nine treatment groups (D through L) for four sensory criteria: Aroma, Color, Taste, and Texture. The scores are based on a 5-point hedonic scale, where 1 indicates the least desirable characteristic (e.g., very fishy aroma, unpleasant taste) and 5 indicates the most desirable characteristic (e.g., not fishy aroma, very pleasant taste). Generally, the control groups exhibit higher mean scores for all characteristics compared to the treatment groups.

Table 2. Organoleptic Characteristics of Chicken Liver Sausages.

Characteristic	Control			Treatment								
	A	B	C	D	E	F	G	H	I	J	K	L
Aroma	4.69	4.73	4.64	4.13	4.16	4.07	4.21	4.3	4.3	4.34	4.37	4.33
Color	4.24	4.06	3.86	2.07	1.94	1.9	2	2	1.81	1.94	2.31	2.26
Taste	4.09	4.03	3.96	2.49	2.69	2.59	2.8	2.93	2.96	3.01	2.99	2.9
Texture	3.56	3.79	3.7	2.9	2.96	2.94	2.99	3.07	2.91	3.24	3.24	3.16

Note: Aroma : 1 (Very fishy), 2 (Fishy), 3 (Fishy), 4 (Moderately fishy), 5 (Not fishy)
Color : 1 (Dark brown), 2 (Brown), 3 (Light brown), 4 (Orange-brown), 5 (Orange)
Taste : 1 (Very unpleasant), 2 (Unpleasant), 3 (Somewhat pleasant), 4 (Pleasant), 5 (Very pleasant)
Texture : 1 (Very chewy), 2 (Chewy), 3 (Not chewy), 4 (Chewy), 5 (Very chewy)

3.1.1. Aroma

In the results of the aroma of sausage products, the highest average result was found for treatment K, with a value of 4.37. This value in the organoleptic test characteristics indicates that the aroma of the sausages is quite fishy. The fishy aroma in the sausages is influenced by each addition of chicken liver; the fishy smell can be reduced by adding spices (Zuraida & Angraini, 2024).

3.1.2. Color

In the color of the sausages product, the highest result was found with an average of 2.31, namely treatment K, which has brownish-orange organoleptic characteristics. In the research of Herlina and Rusdianto (2015), it was stated that the color resulting from the sausages-making process causes the Maillard reaction. The light brown color that affects this sausage product can also be influenced by the myoglobin content, which is a pigment in meat muscle derived from protein (Apriantini, Afriadi, Febriyani, & Arief, 2021).

This is evidenced by previous research by Jokanović et al. (2014), namely that differences in brightness levels in each experiment were influenced by protein-fat interactions and low emulsion stability, which would affect the emulsification process during the production of meat sausages with added chicken liver.

3.1.3. Taste

The taste of chicken liver sausage products has the highest average score of 3.01, specifically in treatment J, which exhibits good to very good characteristics. The taste is not entirely good to somewhat good because some panelists in this study do not find the taste of sausages with added chicken liver to be satisfactory. In other research, it is stated that increasing the amount of chicken liver substitution in a food product can lead to a bitter taste, making it less preferred by panelists (Fauziah, Fajri, & Hermanto, 2019).

3.1.4. Texture

In the texture of chicken liver sausages, the highest average value of 3.24 was observed in treatment J. The chewy characteristic of the sausages indicates that the addition of carrageenan influences the texture. Making sausages using egg whites results in dense or compact sausages. Tapioca flour used has the ability to improve the gel, thereby enhancing the texture of the sausages (Zuraida & Angraini, 2024). The addition of non-meat/plant-based protein ingredients to chicken liver sausage affected the texture and hardness of the sausage, which was not better than the treatment using non-meat ingredients (Pagthinathan & Gunasekara, 2021).

3.2. The Selected Formula

The best formula in this study was determined using the De Garmo method. The results obtained from De Garmo's calculation, based on proximate content and organoleptic properties, indicated that formula J (32.5, 17.5; 15; 1%) was optimal, with an overall NH value of 0.86. Therefore, sausages with the addition of chicken liver, red beans, skim milk, and carrageenan are recommended for treatment in formula J (A3K1). The nutritional content of this treatment is expected to meet the needs of pregnant women who are still lacking in nutrient intake. Additionally, the organoleptic test results show that the sausages are brown to dark brown in color, have a good taste, a slightly fishy aroma, and a texture that is slightly chewy to chewy.

3.3. Chemical Characteristics of Sausages

3.3.1. Ash Content

Ash content in the test results of sausages adding chicken liver for all treatments experienced significant increases and decreases. The ash content of each treatment ranged from 2.11% to 3.11%. The ash content in formula J (A3K1) has the highest value of 3.11±0.02%. It had a significant effect with *p*-value < 0.05. The high and low ash content of a product can be influenced by the food ingredients used (Palandeng, Mandey, & Lumoindong, 2016).

3.3.2. Moisture

All treatments are in accordance with the quality requirements of Indonesian national standards called SNI 3820-2015, which has a maximum of 67%. The moisture content in formula J (A3K1) has the lowest value of 55.31±0.02%. In this research on sausages, the moisture content continues to decrease. It had a significant effect with a *p*-value < 0.05. The high moisture content increases the formulation of wheat flour in sausage making, which is reduced due to TGase substitution and affects the high moisture in the P0 treatment (Ismanto et al., 2020). A high moisture content also increases the risk of product deterioration due to biological activity or the entry and rapid growth of bacteria (Salmahaminati, 2025).

Table 3 presents the chemical characteristics of sausages enriched with chicken liver, kidney beans, and skim milk, displaying the results for twelve different samples (A through L). The data for all chemical parameters, which include Ash (%), Moisture (%), Carbohydrate (Carbo) (%), Protein (%), Fat (%), Calories (kcal), Iron (mg), and Calcium (mg), are reported as Mean ± SD. The statistical analysis, determined by the Welch ANOVA, indicates that the p-value for all measured characteristics is less than 0.001, signifying statistically significant differences among all samples

Table 3. Chemical characteristics of sausages enriched with chicken liver, kidney beans, and skim milk.

Sample	Ash (%)	Moisture (%)	Carbo (%)	Protein (%)	Fat (%)	Calori (kcal)	Iron (mg)	Calcium (mg)
A	2.71±0.02	61.95±0.58	14.79+0.03	14.66±0.02	5.57±0.03	167.90±0.26	3.88±0.03	23.99±0.13
B	2.40±0.02	61.84±0.03	15.82±0.02	14.55±0.02	5.38±0.03	169.93±0.12	3.85±0.02	23.30±0.13
C	2.11±0.02	61.30±0.04	16.94±0.03	14.38±0.03	5.26±0.04	172.65±0.29	3.79±0.02	22.65±0.15
D	2.91±0.02	59.92±0.03	15.65±0.03	16.64±0.03	4.87±0.04	173.04±0.37	6.72±0.03	36.98±0.29
E	2.71±0.02	59.62±0.03	16.77+0.01	16.38±0.02	4.51±0.02	173.22±0.22	6.89±0.04	36.20±0.08
F	2.58±0.03	59.02±0.03	18.00+0.06	16.01±0.02	4.39±0.02	175.55±0.33	7.19±0.02	36.16±0.11
G	2.90±0.01	57.79±0.02	15.36+0.02	17.82±0.03	6.13±0.02	187.89±0.02	8.91±0.02	38.94±1.80
H	2.81±0.02	56.67±0.54	16.63+0.04	17.52±0.03	6.07±0.02	191.23±0.35	8.76±0.03	39.54±0.05
I	2.68±0.03	55.88±0.02	18.22+0.01	17.25±0.05	5.97±0.02	195.58±0.23	8.33±0.02	38.78±0.12
J	3.11±0.02	55.31±0.02	16.16+0.06	18.81±0.02	6.61±0.02	199.35±0.21	9.27±0.02	45.25±0.08
K	2.91±0.02	55.10±0.02	17.19+0.04	18.41±0.02	6.39±0.02	199.91±0.28	9.14±0.03	44.83±0.14
L	2.82±0.03	54.61±0.02	18.13+0.04	18.21±0.02	6.19±0.02	201.08±0.14	9.03±0.03	43.62±0.13
p-value	<0.001*W	<0.001*W	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*W

Note: - Mean±SD; *sig. level = 0.05; w: Welch ANOVA.
- Values with different superscripts in the same column are statistically significantly different (p ≤ 0.05)
- *Indonesian National Standards (SNI 3820-2015).

3.3.3. Calories

Calories in formula J (A3K1) have results of sausages with high calories of 199.35±0.21 (100 g). While it had a significant effect with a *p*-value < 0.05. The energy needs of pregnant women increase with advancing gestational age, rising by 180 kcal in the first trimester and 330 kcal in the second and third trimesters. This increase is necessary to support the optimal growth of the fetus, placenta, and other maternal tissues, as well as to meet the heightened energy demands, with approximately 50% of the energy used for metabolism during pregnancy and fetal metabolic rate (Most, Dervis, Haman, Adamo, & Redman, 2019).

Table 4 presents the quality requirements for meat sausages, referencing Indonesian National Standard Number 3820, year 2015. The table specifies two main test criteria: Protein (measured as N x times 6.25) and Fat. Protein content is required to be a minimum of 13% (weight/weight) for "Sausages Meat" and a minimum of 8% (weight/weight) for "Combination Sausages Meat." Conversely, the fat content must not exceed 20% (weight/weight) for both categories of meat sausages.

Table 4. Quality requirements for meat sausages.

Test criteria	Unit	Terms	
		Sausages Meat	Combination Sausages Meat
Protein (N x 6.25)	% (b/b)	Min. 13	Min.8
Fat	% (b/b)	Max. 20	Max. 20

Source: SNI 3820-2015 Meat Sausages.

3.3.4. Carbohydrate

The highest carbohydrate content, based on the average results of sausage carbohydrates, ranges from 14.79% to 18.22% (per 100 g). The carbohydrate content in the test results was 16.16±0.06% in treatment formula J (A3K1). This had a significant effect with a *p*-value < 0.05. This aligns with research indicating that carbohydrate levels decrease as protein, moisture, ash, and fat levels increase (Herlina, Aji, & Purnomo, 2021).

3.3.5. Protein

The protein in formula J (A3K1) in sausages with the addition of chicken liver, red bean flour, and skimmed milk in treatments has a high value of 18.81±0.02%. It had a significant effect with a *p*-value < 0.05. The range of fat content still fulfills SNI 3820-2015 Combination Meat Sausages, which is at least 8%. This is in accordance with the research of Rahmawati et al. (2025) that the protein content of the sausage combination of chicken liver and tuna fish increased and was in accordance with the high protein requirement of 48% (100g) for children aged 1-3 years. In Lutfiah et al. (2021) research, it is known that formula F4 has a high protein content of 17.21% or 24.6g (per 100g) of chicken liver sausage with the addition of soybean flour, which can meet 10-15% of protein needs. The addition of chicken liver or beef liver can increase the protein content by 5-10% more than the control (Mehmood, Mujahid, Asghar, ur Rahman, & Khalid, 2024).

3.3.6. Fat

The fat content in formula J (A3K1) was influenced by the higher percentage of chicken liver and skimmed milk compared to other treatments. The highest fat content of sausages was 6.61±0.02%. It had a significant effect with a p-value < 0.05. The range of fat content still fulfills SNI 3820-2015 for Combination Meat Sausages, which is below 20%. Too high fat content is not good for the appearance of the sausages, and the emulsion of fat can break down when the heating temperature is higher, which will reduce the fat and moisture content (Ismanto et al., 2020).

3.3.7. Iron

Iron levels contained in the test results show the highest value of 9.27±0.02% in formula treatment J (A3K1). This had a significant effect with a p-value < 0.05. In each food addition, there is a significant increase and a decrease. The increase in each treatment can also be influenced by the proportion of chicken liver, which is 15.8 mg/100 g, red beans at 3.7 mg/100 g, and skimmed milk at 0.6 mg/100 g, indicating higher iron content. Adequate iron consumption in pregnant women can prevent anemia, help boost the immune system, and support fetal growth and development (Febry et al., 2023). The addition of 25% chicken liver is in accordance with the research of Taqiyyah, Zaman, Citra, and Aini (2019) that the Fe content with a ratio of 25% moringa leaves: 25% chicken liver is much greater.

3.3.8. Calcium

Calcium levels in the test results of sausages adding chicken liver for treatment J (A3K1) have a the highest value amounted to 45.25±0.08%. While it had a significant effect with a p-value is important in fulfilling the mineral requirements of pregnant women, especially for fetal development. Calcium deficiency over a long period can result in suboptimal bone mass growth (Zuraida & Angraini, 2024). It is known that 100 g of chicken liver contains 118 mg of calcium, 100 g of kidney beans contains 502 mg of calcium, and 100 g of skimmed milk contains 1300 mg of calcium (Ministry of Health Directorate General of Public Health, 2018).

Table 5. Physical test results of sausages enriched with chicken liver, red bean, and skim milk.

Sample Code	J1 (A3K1)	J2 (A3K2)	J3 (A3K3)	Average	* SNI
Escherichia coli (APM/g)	Negative	Negative	Negative	Negative	Negative (< 3)
Salmonella Sp (APM/g)	Negative	Negative	Negative	Negative	Negative
Chewiness (gf)	1.003.87	997.74	999.17	1.000.26	-
WHC (%)	1.89	1.91	1.93	1.91	-
Cooking loss (%)	4.69	4.73	4.75	4.72	-

Note: *SNI: Standar Nasional Indonesia (Indonesian National Standard), referring to SNI 3820:2015
WHC: Water holding capacity.

3.3.9. Water Holding Capacity (WHC)

The statistical test results had a significant effect (p<0.05) on the WHC of the sausages. Table 5 summarizes the main experimental average results of the WHC test on these sausages, which is 1.91%. The level of WHC is higher when the percentage of chicken liver is added. According to Lenzun, Sompie, and Siswosubroto (2021), the increase in WHC is influenced by the binding of proteins in gelatin to water molecules. The increase in WHC affects the firm texture of sausages with chicken liver and gizzard additives due to the binding of protein additives that bind water content in the sausages (El-Sayed, Farag, & El-Sayed, 2018).

3.3.10. Chewiness

The statistical test results had a significant effect ($p<0.05$) on sausages elasticity. Table 5 summarizes the main experimental average results of the chewiness test on these sausages were adding chicken liver for treatment J (A3K1) has a value of 1.000.26 (gf).

The level of elasticity will increase according to the addition of the percentage of carrageenan. This is evidenced in the research of Indarti and Ismawati (2019), which states that there are several treatments with little addition of carrageenan that have no effect on elasticity, so other ingredients that affect elasticity are tapioca flour. Therefore, the chewiness of the sausages is influenced by the addition of other ingredients such as tapioca flour and eggs, so it is not only caused by the addition of carrageenan.

3.3.11. Cooking Loss

The statistical test results had a significant effect ($p<0.05$) on the cooking loss of sausages. Table 5 summarizes the main experimental average results. The test on these sausages shows a value of 4.72%. In this study, the results of cooking loss increased and decreased insignificantly. This is influenced by the ingredients of chicken meat and the addition of chicken liver.

In the research, the results of 0% beef sausages have a higher shrinkage value than 5% and 10%, and the addition of 15% gelatin has the lowest shrinkage value, so the addition of gelatin level affects the low cooking loss value (Lenzun et al., 2021).

3.3.12. Escherichia Coli and Salmonella Sp. Test

The results of the E. coli and Salmonella sp. test on the best sausages formula treatment J were declared negative. This is in accordance with the provisions of SNI 3820-2015, which specify quality requirements for negative sausages from E. coli and Salmonella.

While both had a significant effect ($p<0.05$). The negative results of both tests can be influenced by the ingredients used and heating or during the cooking of the sausages the bacteria die. The sausages cooking factor can be steamed for 30-45 minutes. In the steaming process, to kill microbes, which is done by the Coldest Point temperature method (Sejati & Wahyuni, 2023).

4. CONCLUSIONS

This study has limitations, in that only in vitro tests were conducted, focusing on physicochemical, microbiological, and organoleptic tests without any in vivo tests or clinical studies. The addition of chicken liver, red bean flour, skim milk, and carrageenan to chicken sausage formulations yielded significant results that improved the physicochemical and organoleptic characteristics of the sausage, particularly in treatment A3K1 (J). With a p value <0.05, there were significant differences in each treatment.

The best formulation also met the quality and safety standards in Indonesian National Standards of SNI 3820-2015. Further research is needed based on mechanisms that include in vitro and in vivo evaluations or clinical studies as well as shelf-life studies to validate the benefits of chicken liver sausage for pregnant women. Broader application can be explored by combining chicken liver with other nutrient-rich ingredients and developing similar formulations, thereby expanding its potential contribution to improving maternal nutrition and reducing the risk of stunting.

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