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Extraction, Characterization, Amino Acid Profile of Halal Gelatin from Kampong and Broiler Chicken Feet Skin

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Abstract

Gelatin is one of the food additives where the issue of halal is interesting to study. Several previous studies have explored alternatives to halal gelatin. This study analyzes gelatin's chemical and physical properties from kampong and broiler chicken feet skin. The results obtained can be utilized to develop a halal alternative to gelatin. The study used a nested randomized design consisting of two factors: variations in the chicken origin and the concentration of the acid solvent. The first factor, the kind of chicken, had two levels, kampong and broiler chickens. The second factor was acetic acid concentration (CH_3COOH) with 2 %, 4 %, 6 %, and 8 % (v v^{-1}). Gelatin from the skin of kampong chicken feet skin with 4 % (v w^{-1}) acetic acid reached a yield of 12.67 %, a moisture content of 10.29 %, an ash content of 1.58 %, a protein content of 82.52 %, a pH 4.53, a viscosity of 4.78 cp, and a gel strength of 66.29 g cm^{-2}. Gelatin from broiler chicken leg skin with 4 % acetic acid reached a yield of 10.90 %, a moisture content of 7.95 %, an ash content of 7.95 %, a protein content of 82.48 %, a pH of 4.76, a viscosity of 5.4 cp, and a gel strength of 70.13 g cm^{-2}. Kampong and broiler chicken feet skin gelatin were similar to commercial gelatin concerning the glycine percentage. Glycine has more than 50 % gelatin from all amino acids. Glycine from kampong chicken feet skin gelatin was 53.37 %, broiler chicken feet skin gelatin had 51.95 %, and commercial gelatin was 54.33 %. The meatballs using kampong and broiler chicken feet skin gelatin (acetic acid 4 %) meet all the requirements of NSA (National Standardization Agency of Republic of Indonesia -*Standar Nasional Indonesia*: 3818-2014) for moisture, ash, protein, and lipid content variables.

Keywords: Acetic acid hydrolysis, Collagen, Environmentally friendly, *Gallus gallus domesticus* L., Organic acid hydrolysis, Poultry waste, Waste to halal food, Waste utilization

1. Introduction

The poultry processing industry is rapidly developing along with the food industry. Annually, about 69×10^6 t of chicken meat is produced worldwide. FAO data shows that $3\,900 \times 10^3$ t chicken feet were produced from the poultry processing industry (FAO Statistics, 2020). In some countries, chicken feet are thrown away without any further processing, which causes environmental hazards (Dhakal *et al*., 2020; Radhakrishnan *et al*., 2020). Several researchers (Karuppannan *et al*., 2021; Lasekan *et al*., 2013) suggest implementing zero waste in the poultry industry; in addition, the poultry processing industry produces side products such as liver, gizzard, feet, and other innards, and researchers recommend processing this waste into renewable energy, livestock feed, fertilizers, and pet foods. Haghighi *et al*. (2021); Janarthanam *et al*. (2020) have reported using chicken slaughter waste as a source of biodiesel. Some researchers (Adinurani *et al*., 2017; Abdullah *et al*., 2021; Latifi *et al*., 2019; Setyobudi *et al*., 2021a) recycle poultry waste into sustainable clean energy, namely biogas.

Lachenmeier *et al.* (2022) show –with a pyramid model for waste utilization–that food is a primary priority. Gelatin is one of the hydrocolloids obtained from the hydrolysis of collagen from skin, bones, or another animal's part. The gelatin extraction from collagen can be done by chemical and biochemical hydrolysis. Collagen is the main protein in the skin, with a high content of fibrous protein (Liu *et al*., 2015; Mariod and Fadul, 2013). Gelatin is widely used in food, pharmaceuticals, and cosmetics to add nutrients and functional properties, generally derived from porcine or bovine sources. Gelatin, therefore, mainly originates from porcine skin (46 %), bovine skin (29.4 %), and porcine or bovine bone (23.1 %) (Gómez-Guillén *et al*., 2011).

Research on gelatin is currently focusing on finding alternative sources over pig sources due to the Halal issue for Muslims (Rakhmanova *et al*., 2018; Shah and Yusof, 2014). The demand for halal gelatin is reflected by the total number of Muslims worldwide. The Muslim

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population amounts to 23.4 % of the global world population (Jamaludin *et al.*, 2011). There are various alternative sources of gelatin that several researchers have developed, such as seaweed (Nderitu *et al.*, 2011), other mammals (Al-Saidi *et al.*, 2012; Nitsuwat *et al.*, 2021; Sarbon *et al.*, 2013; Tümerkan, 2021), fish (Alfaro *et al.*, 2015; Zhang *et al.*, 2015), insect (Mariod and Fadul, 2013), and also poultry (Chakka *et al.*, 2017; Widyasari and Rawdkuen, 2014).

Research from poultry on gelatin has been performed on bird feet (Lin and Liu, 2007), silky fowl feet (Martínez-Ortiz *et al.*, 2015), chicken meat residues (Ramaya *et al.*, 2022), chicken skin (Silva *et al.*, 2021), and chicken feet (Chakka *et al.*, 2017; Hlaing *et al.*, 2020; Widyasari and Rawdkuen, 2014). The use of chicken feet, thus, is a considerable alternative raw material for halal gelatin. In addition, previous research stated that there is 18 % protein in chicken feet (Suparno and Prasetyo, 2019), showing sufficient potential as an alternative raw material for halal gelatin.

The most frequently consumed chickens in Indonesia are broilers and kampongs (native) chicken (*Gallus gallus domesticus* L.). Broiler chickens are pure-bred chickens having fast growth rates. In broilers, reaching a finishing weight of 1.5 kg takes a shorter time of 5 wk to 7 wk only, while kampong chicken requires a longer time of 30 wk to obtain a weight of 1.5 kg. Broiler chickens, thus, have a shorter economically relevant lifespan than kampong chickens. When slaughtered at an old age and take part of the muscle to take the collagen, we will get more collagen protein content because muscle tissue mainly consists of collagen (Cahyono, 2011). Differences in muscle tissue composition and collagen content in livestock relate to quality differences in gelatin because gelatin is obtained from the hydrolysis of collagen.

Gelatin is thus obtained by extraction, a chemical hydrolysis process with acids or bases (Das *et al.*, 2017). An acidic extraction process can change the basic structure of collagen from a triple helix into a single helix structure through a different extraction process than the one in an alkaline environment. It can only change the basic structure from a triple helix into a double helix, so the extraction of gelatin using acidic hydrolysis takes a shorter time than alkaline hydrolysis. The type of acid used in this study is an organic acid, namely acetic acid (CH_3COOH). Using inorganic acids such as hydrochloric acid (HCl) produces a very pungent odor, and the resulting gelatin is also darker in color (Liu *et al.*, 2015).

A previous study producing gelatin from the extraction of chicken feet using acetic acid (CH_3COOH), citric acid ($C_6H_8O_7$), and lactic acid ($C_3H_6O_3$) from different concentrations also evaluated its physicochemical properties (Chakka *et al.*, 2015). Other studies have produced physicochemical comparisons between gelatin made from chicken feet and gelatin made from bovine (Rahman and Jamalulail, 2012; Sarbon *et al.*, 2013). The present study aims to extract the gelatin from chicken feet skin from two chicken types, broiler chicken and kampong chicken, using acetic acid (CH_3COOH) in 2 %, 4 %, 6 %, and 8 % (v w^{-1}). The authors used acidic extraction to obtain gelatin and then analyzed the yield, moisture content, protein content, viscosity, and gel strength. The results could be used as a reference for producing gelatin from halal ingredients. The amino acid from the extracted gelatin compared to commercially available gelatin was analyzed using High-Performance Liquid Chromatography (HPLC). The best product from the extracted gelatin samples was also used to make meatballs.

2. Materials and Methods

2.1. Materials

Fresh chicken feet were obtained from Malang, East Java, Indonesia. Chemicals for this study include acetic acid (CH_3COOH), sulfuric acid (H_2SO_4) 98 %, Na_2SO_4-HgO catalyst, sodium hydroxide (NaOH) 50 %, boric acid ($HBrO_3$) 4 %, hydrochloric acid (HCl) 0.02 N, and petroleum benzene. All chemicals in this study used Pro Analytic, Merck.

2.2. Methods

First, chicken feet were washed to remove impurities and then boiled at 80 °C for 10 min. Next, chicken feet that had been sliced were rewashed to remove the remaining fat. Chicken feet were then skinned, cut into 2 cm-sized pieces, and weighed as much as 60 g. The skin was soaked with a solution of acetic acid (CH_3COOH), concentration 2 %, 4 %, 6 %, and 8 % (v w^{-1}) for 24 h. The immersion process brought up ossein. Ossein was then washed with distilled water until the pH was between 5 to 6. Ossein was extracted with distilled water (1:2) at 70 °C (Biobase WD-AD5, China) for 4 h to produce the gelatin solution. The gelatin solution was filtered (IKEME, type IKEM-5, China) and poured into a baking pan (Maspion, Indonesia). The baking sheet was put in the refrigerator (Hitachi R-VX40PGD9, Japan) at 5 °C for 24 h to concentrate the gelatin solution until the formation of the gel. The gelatin gel was dried in a cabinet dryer (Aneka Mesin, AM-TD24, Indonesia) at 60 °C for 78 h. Dry gelatin was mashed to powder (Santana *et al.*, 2020).

2.3. Characterization of gelatin

The extracted gelatin was analyzed for its amino acid composition using HPLC at the IPB *(Institut Pertanian Bogor)* University Laboratory, Indonesia. Samples of gelatin were injected into the HPLC column. HPLC column details: 150 mm × 4.6 mm ODS-2 Hypercell column and fluorescence detectors were used (Shimadzu, LP-32-IDN, Japan). Characterization of gelatin was carried out by separation through the conditions were set at a flow rate of 1 mL min^{-1} with separation using an eluent gradient system. The mobile phase uses eluent A, a mixture of sodium acetate pH 6.5, sodium ethylenediamine tetraacetate (Na-EDTA), methanol, and tetrahydrofuran (THF), and eluent B, which is a mixture of 95 % methanol and ion-free water (Setyobudi *et al.*, 2021b). Result of characterization of gelatin in the form of quantity (%) of amino acids: Aspartic acid, Threonine, Serine, Glutamate, Proline, Glycine, Alanine, Valine, Methionine, Ileusine, Leusine, Tyrosine, Phenylalanine, Histidine, Lysine, Arginine.

2.4. Application of gelatin

Forming meatballs began with the preparation of ingredients, namely, fresh chicken meat (1 kg), tapioca flour (150 g), garlic (25 g), salt (20 g), pepper (8 g), ice cube (200 g) and gelatin powder (7.5 g). The chicken meat was ground by adding salt and ice cubes. Spices such as garlic, shallot pepper, gelatin powder, and tapioca flour were used during the grinding process. The dough was then printed round and immediately boiled at a temperature of 60 °C to 80 °C. The cooked meatballs floated for about 10 min (Kilic *et al.*, 2021).

2.5. Gelatin and meatball analysis

Broiler and kampong chicken feet skin samples were analyzed as raw materials. The analyses of raw materials included protein, moisture, and ash content. The extracted gelatin was investigated for chemical and physical composition. The proximate analyses consisted of moisture (thermogravimetry, oven, Roman, Switzerland), ash (thermogravimetry, oven, Roman, Switzerland), and protein (Kjeldahl, Genhadet, German) content. The physical analyses consisted of pH (pH meter, SI Lab, United States), viscosity (viscometer, Nesco, Shanghai), and gel strength (texture analyzer, Shimadzu, Japan). A chemical analysis of the meatballs was conducted to test product quality concerning the extracted gelatin. As control samples were used, meatballs with commercially available gelatin and meatballs without adding gelatin. The meatball was analyzed for moisture, ash, protein, fat, texture (NSA-RI, 2019), and organoleptic properties (Damat *et al*., 2021a; NSA-RI, 2006).

As an organoleptic analysis, this study performed a hedonic analysis which was carried out using untrained student panelists to identify consumer preferences for food ingredients. The panel used for the organoleptic test consisted of 24 untrained panelists (Damat *et al*., 2021b; NSA-RI, 2006). The organoleptic properties assessment scores are presented in Table 1.

Table 1. Assessment score on organoleptic analyses of chicken meatballs

Score	Aroma	Taste	Texture	Appearance
1	Strong distaste	Strong distaste	Strong distaste	Strong distaste
2	Distaste Moderately	Distaste moderately	Distase moderately	Distaste moderately
3	Weak distaste	Weak distaste	Light distaste	Light distaste
4	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike
5	Enjoy slightly	Enjoy slightly	Enjoy slightly	Enjoy slightly
6	Enjoy moderately	Enjoy moderately	Enjoy moderately	Enjoy moderately
7	strong preference	strong preference	strong preference	strong preference

2.6. Research design

The study covered the extraction of chicken feet skin gelatin with various types of chicken and different concentrations of acetic acid solvents. The first stage of the experimental design had a randomized design with two factors and three replications. The first factor was the chicken type with two levels, broiler chicken and kampong chicken. The second factor used was the concentration of acetic acid (CH_3COOH) with four levels of 2 %, 4 %, 6 %, and 8 % (v w^{-1}). Duncan's new multiple-range tests were used to determine the means, and $P \leq 0.05$ was considered statistically significant. Processing data using formulas in excel (Adinurani 2016, 2022).

3. Results and Discussion

3.1. Raw material analysis

Raw material analysis was used to pre-screen the protein and moisture content of the raw material. It is important because this study used different chicken types, differences in feed, and finally, differences in slaughtering age, which may have affected the chemical content (Cahyono, 2011). Table 2 shows that the protein and ash content of kampong chicken feet skin were higher than the equivalent numbers in broiler chicken. Similarly, the moisture content of kampong chicken feet skin was lower than that of broilers.

Table 2. Analysis results of raw material for chicken feet skin

Component	Kampong chicken feet skin	Broiler chicken feet skin	Chicken feet (Suparno and Prasetyo, 2019)
Protein (%)	22.73	22.00	18.09
Moisture (%)	63.01	64.00	64.00
Ash (%)	3.50	3.37	3.50

3.2. Yield and characterization of gelatin

Based on the analysis of variance, both the chicken type and acidic concentration significantly affected the yield of the gelatin produced (α = 5 %). The highest gelatin yield was obtained through 4 % acetic acid at extraction (Table 3). The highest yield from kampong chicken feet skin gelatin was 12.67 %, while broiler chicken feet skin was 10.0 %. The results might be explained as the raw material (collagen protein in the skin) was higher in kampong chicken feet than in broiler chicken (Table 1). This yield is even higher than in a previous study using 1.5 % acetic acid (6.59 %), 3 % acetic acid (8.51 %), and 4.5 % acetic acid (10.16 %) (Chakka *et al*., 2017). However, this present study achieved lower results than the previous study, which used alkaline-based sodium hydroxide (NaOH 0.15 %), resulting in 16 % (Sarbon *et al.*, 2013). Extraction with acid solvents effectively hydrolyses collagen through peptide bonds (Sarbon *et al*., 2013). The acid solvent is even capable of dissolving non-cross collagen and of breaking down some cross bonds between strands in collagen, leading to higher solubility (Liu *et al*., 2015).

Table 3. Detailed analysis of chicken feet skin gelatin

Treatment		Dry based				Gel based		
Chicken type	Concentration of acetic acid (%)	Yield (%)	Moisture (%)	Ash (%)	Protein (%)	pH	Viscosity (cp)	Gel strength (g cm^{-2})
Kampong chicken feet skin	2	10.01 ± 0.04 ^{bc}	11.98 ± 0.47 ^{d}	1.67 ± 0.31 ^{b}	80,25 ± 0.33 ^{b}	5.49 ± 0.03 ^{d}	4.56 ± 0.04 ^{b}	63.47 ± 0.30 ^{b}
	4	12.67 ± 0.27 ^{d}	10.29 ± 0.14 ^{c}	1.58 ± 0.27 ^{b}	82,52 ± 0.75 ^{ab}	4.53 ± 0.08 ^{c}	4.78 ± 0.09 ^{bc}	66.29 ± 0.43 ^{c}
	6	11.36 ± 0.33 ^{c}	8.76 ± 0.98 ^{bc}	1.54 ± 0.31 ^{b}	77,21 ± 0.07 ^{ab}	4.14 ± 0.01 ^{b}	4.21 ± 0.02 ^{ab}	61.43 ± 0.28 ^{b}
	8	10.44 ± 0.63 ^{c}	7.54 ± 0.50 ^{b}	1.37 ± 0.60 ^{b}	76,28 ± 0.59 ^{a}	3.33 ± 0.06 ^{a}	4.02 ± 0.02 ^{a}	59.61 ± 0.05 ^{a}
Broiler Chicken Feet Skin	2	7.05 ± 0.10 ^{a}	9.48 ± 0.49 ^{c}	0.87 ± 0.01 ^{ab}	81,29 ± 0.26 ^{b}	5.64 ± 0.03 ^{d}	5.23 ± 0.02 ^{cd}	67.49 ± 0.22 ^{bc}
	4	10.90 ± 0.10 ^{c}	7.95 ± 0.40 ^{bc}	0.58 ± 0.19 ^{ab}	82,48 ± 0.96 ^{b}	4.76 ± 0.09 ^{c}	5.44 ± 0.02 ^{d}	70.13 ± 0.48 ^{c}
	6	9.10 ± 0.11 ^{b}	6.80 ± 0.12 ^{ab}	0.47 ± 0.13 ^{ab}	79,07 ± 0.95 ^{ab}	4.25 ± 0.01 ^{bc}	4.98 ± 0.05 ^{c}	64.95 ± 0.12 ^{b}
	8	9.42 ± 0.77 ^{bc}	5.52 ± 0.04 ^{a}	0.27 ± 0.21 ^{a}	77,63 ± 0.62 ^{ab}	4.02 ± 0.08 ^{b}	4.72 ± 0.08 ^{bc}	62.02 ± 0.74 ^{ab}

The average value followed by the same letter indicates no significant effect according to Duncan's test α = 5 %

3.2.1. Moisture content

The analysis of variance showed that the chicken type and treatment had a very significant effect (α = 5 %). The concentration of acetic acid substantially affected the moisture content of chicken feet skin gelatin. Table 3 shows that the mean moisture content of chicken feet skin gelatin produced in this study ranged between 5.52 % and 11.99 %. The moisture content of chicken feet skin gelatin obtained in the study met the GMIA (2012) and requirements, a maximum of 16 %. The average moisture content of broiler chicken feet gelatin was lower than in kampong chicken. The moisture content range in kampong chicken gelatin was between 7.54 % and 11.98 %, while that of broiler chicken feet skin gelatin was between 5.52 % and 9.48 %. The range relates to the protein content in the raw material (Table 2). Chicken feet skin from broilers had a lower protein content than kampong chicken. Broiler chicken feet skin had a protein content of 22.00 %, which amounted to 22.73 % in kampong chicken. The protein content in chicken feet skin could bind water and the added water from the processing process. That figure is called the water-holding capacity (WHC) (Bowker and Zhuang, 2015).

3.2.2. Ash content

The analysis of variance also revealed that the chicken type related very significantly (α = 1 %) to the ash content of the feet skin gelatin. Manipulating the acetic acid concentration did not substantially affect the gelatin ash content of chicken feet skin. The average ash content range of chicken feet skin gelatin produced in this study was 0.55 % to 1.54 %. The ash content of chicken feet skin gelatin obtained in this study has met the GMIA requirements, as the maximum allowed ash content in gelatin is 3.35 %. A demineralization process with acetic acid at a concentration of 2 % to 8 % for 24 h can liberate minerals from raw materials to produce gelatin with low ash content. The mean gelatin ash content from broiler chicken feet skin (0.55 %) was lower than that of kampong chicken (1.54 %). Therefore, the concentration of acetic acid had no significant effect on the gelatin ash content of chicken feet skin. Still, the average ash content of chicken feet skin gelatin decreased with increasing acetic acid concentration (Weng *et al*., 2014). Ash content in gelatin indicates the presence of minerals present in it. Gelatin from chicken feet is known for containing several macro- (Na, Ca, K, Mg, P, S) and microminerals (Cu, Fe, Mn) (Santana *et al.*, 2020).

3.2.3. Protein content

Analyses of variance showed that chicken types and acetic acid concentration had a very significant effect (α = 5 %) on the protein content of chicken feet skin gelatin. The average protein content of chicken feet skin gelatin in both chicken types and the concentration of acetic acid are presented in Table 2. The mean percentage of kampong chicken gelatin protein content ranged between 82.28 % and 80.25 %, while broiler chicken feet skin ranged between 82.48 % and 77.63 %. The highest protein content in the study was found when 4 % acetic acid was used with village chickens' skin (82.52 %) and broilers (82.48 %). Using the acid extraction method, the protein content in gelatin produced from the chicken feet skin and broilers was similar to previous studies (Chakka *et al*., 2017). Acid extraction resulted in higher protein contents than in alkaline environments and beef samples. The protein content of gelatin from chicken feet with alkaline extraction amounted to 80.76 %, while gelatin from cows contained 81.75 % (Sarbon *et al.*, 2013). The low protein content of kampong chicken feet skin gelatin compared to broiler chicken feet skin gelatin was most likely caused by the relatively high ash content. The high ash was considered a mineral residue that might inhibit protein binding to reactive groups in gelatin such as -OH, -COOH, and $-NH_2$ so that the intermolecular binding of gelatin proteins becomes weak (Gómez-Guillén *et al*., 2011).

3.2.4. pH

The chicken type had no significant effect (α = 5 %) on chicken feet skin gelatin's pH (acidity). However, the acetic acid concentration significantly affected chicken feet skin gelatin's pH (degree of acidity). Although the average pH was not much different between boiler chicken feet skin gelatin and kampong chicken, boiler chicken feet skin gelatin had a slightly higher pH of 4.35, while kampong chicken feet skin gelatin was 4.33.

3.2.5. Viscosity

Chicken type and acetic concentration had a very significant effect (α = 5 %) on the viscosity of chicken feet skin gelatin. Table 2 shows that the mean viscosity of chicken feet skin produced in this study ranged between 4.02 cp and 5.44 cp. The viscosity of chicken feet skin gelatin met the requirements of GMIA (2012), requiring a viscosity in gelatin between 1.5 cp and 7.5 cp. The protein in gelatin had a high water binding power to protein or was hydrophilic. Broiler chicken skin gelatin had a higher protein content, so its ability to bind water was also higher (Table 2) to reduce moisture and increase gelatin viscosity. Based on the Duncan test (α = 5 %), the acetic acid concentration significantly affected the resulting chicken feet skin gelatin viscosity. The viscosity of gelatin in chicken feet skin decreased with increasing concentration of the acetic acid solution. The lowest viscosity was found at an acetic acid concentration of 8 % in the gelatin of kampong and broiler chicken feet and amounted to 4.02 cp and 4.72 cp, respectively. Acetic acid can break the peptide bonds of amino acids into shorter molecular chains to decrease their viscosity. The number of amino acid chains and their molecular weight are directly proportional to viscosity (Sompie *et al.*, 2015, Sompie and Triasih, 2018).

3.2.6. Gel strength

This study observed a very significant effect (α = 5 %) of chicken species on gelatin strength from chicken feet skin. Changing the concentration of acetic acid substantially affected the gelatin gel's strength. The average strength of chicken feet skin gel produced in this study was (59.61 to 70.13) g cm^{-2}. The power of gelatin from chicken feet skin in this study met the requirements of GMIA since the strength of the gel in gelatin has to be in the range of (50 to 300) g cm^{-2}. Based on the Duncan test (α = 5 %), the chicken species had a powerful effect on gelatin gel strength. Table 2 shows that the average gelatin strength of broiler chicken feet skin was higher than that of kampong chickens. The average power of gelatin from broiler chicken feet skin produced in this study was (62.02 to 70.13) g cm^{-2}, while the kampong chicken was between 59.61 g cm^{-2} and 66.29 g cm^{-2}. Gel strength and gel viscosity are directly proportional to each other. As observed in this study, broiler chicken skin gelatin had a higher viscosity and also a higher gel strength. Glycine, hydroxyproline, and proline are amino acids responsible for maintaining the stability of the gelatin structure. Hydrolysis reactions break down proteins into amino acids and thus increase the distribution of molecules and gel strength (Sompie *et al.,* 2015).

3.3. Amino acid profile

Table 4 details the gelatin amino acid composition of kampong chicken feet skin gelatin from broiler chicken feet skin gelatin and commercially produced (bovine) gelatin. Kampong and broiler chicken feet skin gelatin had a similar percentage of glycine as the commercial gelatin. Glycine makes up more than 50 % of all the amino acids in gelatin. This study observed 53.37 % glycine in kampong chicken feet skin gelatin and 51.95 % in broiler chicken feet skin gelatin compared to 54.33 % in commercial (bovine) gelatin. The second-highest amino acid after glycine was alanine (Table 4). Although the percentages differed significantly, that finding corresponds to previous studies. Alanine in kampong chicken feet skin gelatin was 8.48 %; in broiler chicken feet skin gelatin; it was 8.39 %, and in (bovine) commercial gelatin, it was 7.72 %. Glycine and alanine influence the structure of gelatin and the functional properties of gelatin as an emulsifier (Chakka *et al*., 2017).

Table 4. HPLC analysis of amino acid from kampong, broiler, and commercial gelatin

No.	Amino acid	Kampong chicken feet skin gelatin		Broiler chicken feet skin gelatin		Commercial gelatin (Bovine)	
		RT	% Area	RT	% Area	RT	% Area
1	Aspartic acid	5.78	2.77	7.16	2.73	7.167	2.77
2	Threonine	2.02	2.64	8.73	2.72	8.738	2.31
3	Serine	2.53	2.96	9.37	2.93	9.369	3.75
4	Glutamate	11.90	5.95	10.54	5.81	10.542	5.58
5	Proline	11.881	3.83	12.00	3.82	12.007	3.87
6	Glycine	32.91	53.37	15.86	51.95	15.871	54.33
7	Alanine	11.16	8.48	17.19	8.39	17.194	7.72
8	Valine	1.81	2.30	22.13	2.36	22.117	2.76
9	Methionine	0.77	0.46	23.35	0.42	23.344	0.32
10	Ileusine	1.23	1.24	25.53	1.22	25.526	1.51
11	Leusine	3.03	1.86	26.26	1.84	26.248	1.82
12	Tyrosine	0.42	0.11	27.73	0.10	27.727	0.07
13	Phenylalanine	2.54	1.39	28.84	1.33	28.831	1.17
14	Histidine	0.90	0.94	30.42	1.00	30.410	0.98
15	Lysine	4.08	4.29	32.30	4.24	32.293	3.74
16	Arginine	8.02	5.32	35.84	7.09	35.823	5.41

3.4. Determination and best treatment

The best treatment was determined by comparing the products with the GMIA standard (Table 5). It can be seen in Table 5, the use of acetic acid at a concentration of 4 % gave the highest yield in kampong (12.67 % ± 0.27 %) and broilers chicken feet skin gelatin (10.90 % ± 0.10 %). Besides that, gelatin that used 4% acetic acid shows moisture, ash, pH, viscosity, gel strength, colour, aroma, and also taste that according to the GMIA standard. Meanwhile, protein of gelatin from 4 % acetic acid kampong and broiler chicken feet skin closest to the standard value.

Table 5. Comparison of obtained gelatin characteristics with GMIA (standard)

Treatment		Dry based				Gel based				
Chicken type	Concentration of acetic acid (%)	Yield (%)	Moisture (%)	Ash (%)	Protein (%)	pH	Viscosity (cp)	Gel strength (g cm^{-2})	Colour	Aroma, taste
Kampong chicken feet skin	2	10.01 ± 0.04 ^{bc}	11.98 ± 0.47 ^{d}	1.67 ± 0.31 ^{b}	80,25 ± 0.33 ^{b}	5.49 ± 0.03 ^{d}	4.56 ± 0.04 ^{b}	63.47 ± 0.30 ^{b}	Yellowish	Normal
	4	12.67 ± 0.27 ^{d}	10.29 ± 0.14 ^{c}	1.58 ± 0.27 ^{b}	82,52 ± 0.75 ^{ab}	4.53 ± 0.08 ^{c}	4.78 ± 0.09 ^{bc}	66.29 ± 0.43 ^{c}	Yellowish	Normal
	6	11.36 ± 0.33 ^{c}	8.76 ± 0.98 ^{bc}	1.54 ± 0.31 ^{b}	77,21 ± 0.07 ^{ab}	4.14 ± 0.01 ^{b}	4.21 ± 0.02 ^{ab}	61.43 ± 0.28 ^{b}	Yellowish	Normal
	8	10.44 ± 0.63 ^{c}	7.54 ± 0.50 ^{b}	1.37 ± 0.60 ^{b}	76,28 ± 0.59 ^{a}	3.33 ± 0.06 ^{a}	4.02 ± 0.02 ^{a}	59.61 ± 0.05 ^{a}	Yellowish	Normal
Broiler Chicken Feet Skin	2	7.05 ± 0.10 ^{a}	9.48 ± 0.49 ^{c}	0.87 ± 0.01 ^{ab}	81,29 ± 0.26 ^{b}	5.64 ± 0.03 ^{d}	5.23 ± 0.02 ^{cd}	67.49 ± 0.22 ^{bc}	Yellowish	Normal
	4	10.90 ± 0.10 ^{c}	7.95 ± 0.40 ^{bc}	0.58 ± 0.19 ^{ab}	82,48 ± 0.96 ^{b}	4.76 ± 0.09 ^{c}	5.44 ± 0.02 ^{d}	70.13 ± 0.48 ^{c}	Yellowish	Normal
	6	9.10 ± 0.11 ^{b}	6.80 ± 0.12 ^{ab}	0.47 ± 0.13 ^{ab}	79,07 ± 0.95 ^{ab}	4.25 ± 0.01 ^{bc}	4.98 ± 0.05 ^{c}	64.95 ± 0.12 ^{b}	Yellowish	Normal
	8	9.42 ± 0.77 ^{bc}	5.52 ± 0.04 ^{a}	0.27 ± 0.21 ^{a}	77,63 ± 0.62 ^{ab}	4.02 ± 0.08 ^{b}	4.72 ± 0.08 ^{bc}	62.02 ± 0.74 ^{ab}	Yellowish	Normal
GMIA (standard)		-	Max 16	Max 16	87.26	4.5 to 6.5	1.5 to 7.5	50 to 300	Yellowish	Normal

Gelatin from kampong and broiler chicken feet skin (acetic acid 4 %) was used to produce meatballs and compare them with control meatballs consisting of commercial gelatin and meatballs without any gelatin (Table 6). Gelatin is recognized as a food additive that acts as a thickening agent. Therefore, gelatin quality can affect the meatball quality, especially the texture (Hafid *et al*., 2020). This study's results on moisture, ash, protein, fat, and texture can be seen in Table 6. In addition, the organoleptic (aroma, taste, texture, appearance) variables can be obtained from Table 7. Based on the data in Table 6, it can be interpreted that the meatballs using kampong and broiler chicken feet skin gelatin (acetic acid 4 %) were meeting the NSA (National Standardization Agency of Republic of Indonesia-*Standar Nasional Indonesia*: 3818-2014 (NSA – RI, 2019) on all variables including moisture, ash, protein, and lipids.

Table 6. Results of meatball composition

Meatball type	Proximate analysis				
	Moisture content (%)	Ash content (%)	Protein content (%)	Lipid content (%)	Texture (N mm^{-2})
Using kampong chicken feet skin gelatin (Acetic acid 4 %)	68.08±0.02 ^{c}	1.91±0.03 ^{d}	9.43±0.02 ^{b}	2.80±0.02 ^{d}	19.80±0.01 ^{b}
Using broiler chicken feet skin gelatin (Acetic acid 4 %)	66.93±0.03 ^{b}	1.42±0.04 ^{c}	10.40±0.02 ^{c}	2.09±0.02 ^{b}	25.27±0.02 ^{d}
Commercial gelatin (bovine)	65.93±0.02 ^{a}	1.28±0.02 ^{b}	10.83±0.02 ^{cd}	2.41±0.02 ^{c}	24.88±0.02 ^{c}
Without gelatin	69.52±0.04 ^{d}	0.80±0.04 ^{a}	8.95±0.02 ^{a}	1.35±0.02 ^{a}	18.07±0.01 ^{a}
NSA (National Standardization Agency of Republic of Indonesia -Standar Nasional Indonesia: 3818-2014)	max. 70.0	max. 3.0	min. 8.0	max. 10.0	-

The mean value followed by the same letter indicates no significant effect according to Duncan's test $\alpha = 5\%$

Table 7. Test results from organoleptics of meatballs

Meatball type	Organoleptic test			
	Aroma	Taste	Texture	Appearance
Using kampong chicken feet skin gelatin (Acetic acid 4 %)	5.74 [a]	5.27 [ab]	5.04 [b]	3.19 [b]
Using broiler chicken feet skin gelatin (Acetic acid 4 %)	5.75 [a]	5.27 [ab]	6.09 [c]	4.16 [c]
Commercial gelatin (bovine)	5.74 [a]	5.28 [ab]	6.10 [c]	5.06 [d]
Without gelatin	5.74 [a]	5.23 [a]	4.01 [a]	2.33 [a]

The mean value followed by the same letter indicates no significant effect according to Duncan's test $\alpha = 5$ %

Based on Table 6, the meatball moisture content using commercial gelatin was lower than that of meatballs using broiler chicken feet gelatin (65.93 % versus 66.93 %), and meatballs using kampong chicken feet skin gelatin were lower than meatballs without gelatin (68.08 % versus 69.52 %). However, meatballs using broiler chicken had a similar result as those containing commercial gelatin. Thus, it can be concluded that the water binding capacity of both gelatin types was almost the same. Gelatin can form hydrogen with water in foodstuffs. When gelatin binds to water, the gelation rate increases, thereby increasing the chewy texture of the meat (Mariod and Fadul, 2013).

The mineral content data sequentially from lowest to highest were as follows: meatballs without gelatin (0.80 %), meatballs with commercial gelatin (1.28 %), meatballs using broiler chicken feet skin gelatin (1.42 %), and meatballs using kampong chicken feet skin gelatin (1.91 %). The ash content of the meatballs using kampong and broiler chicken feet skin gelatin followed NSA (National Standardization Agency of Republic of Indonesia -Standar Nasional Indonesia).

Like moisture, protein contents showed that meatballs with gelatin using broiler chicken feet skin gelatin (10.40 %) had almost the same value as meatballs with commercial gelatin 10.83 %). That percentage has overcome the minimum required protein standard of NSA (National Standardization Agency of Republic of Indonesia - *Standar Nasional Indonesia*, (minimum 8.0 %). The meatballs using kampong chicken feet skin gelatin (9.43 %) still met NSA standards. The lipid content shown in the meatballs using kampong and broiler chicken feet skin gelatin followed the standard because it did not exceed 10 %.

Aroma and taste (*i.e.*, organoleptic) results from meatballs using kampong and broiler chicken feet skin gelatin showed similar results compared to meatballs using commercial gelatin and meatballs without gelatin. Thus, the use of gelatin did not affect the aroma and taste of the meatball itself. However, the results of the organoleptic tests differed in texture and appearance. The use of gelatin affects the texture and appearance of the meatballs perceived by the panelists. Panelists preferred meatball texture and appearance of meatballs using broiler chicken feet skin gelatin and commercial gelatin over meatballs using kampong chicken feet skin gelatin and meatballs without gelatin.

4. Conclusion

This research paper shows that the feet skin of kampong and broiler chickens has a high potential to become a halal alternative to gelatin. Both chemical and physical variables derived from the GMIA standard support this claim. Using 4 % acetic acid provided the best results in raw materials from the feet skin of kampong and broiler chickens. Gelatin from the skin of kampong chicken feet skin with 4 % acetic acid yielded 12.67 %, moisture content of 10.29 %, ash content of 1.58 %, protein content of 82.52 %, pH 4.53, a viscosity of 4.78 cp, and gel strength of 66.29 g cm^{-2}. Gelatin from broiler chicken feet skin with 4 % acetic acid yielded 10.90 %, a moisture content of 7.95 %, an ash content of 7.95 %, a protein content of 82.48 %, a pH of 4.76, a viscosity of 5.4 cp, and a gel strength of 70.13 g cm^{-2}. In addition, kampong and broiler chicken feet skin gelatin provided similar percentages of glycine as commercial gelatin. Glycine makes up more than 50 % of all amino acids in gelatin. Glycine from kampong chicken feet skin gelatin was 53.37 %, broiler chicken feet skin gelatin 51.95 %, and commercial gelatin (bovine) was 54.33 %. Meatballs using Kampong and broiler chicken feet skin gelatin (acetic acid 4 %) met the requirements of NSA (National Standardization Agency of Republic of Indonesia -*Standar Nasional Indonesia*: 3818-2014) on moisture, ash, protein variables, and lipids. Organoleptic test showed that aroma and taste from meatballs using kampong and broiler chicken feet skin gelatin showed similar results compared to meatballs using commercial gelatin and meatballs without gelatin; panelists preferred meatball texture and appearance of meatballs using broiler chicken feet skin gelatin and commercial gelatin over meatballs using kampong chicken feet skin gelatin and meatballs without gelatin.

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