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Production and Physical Quality Testing of Gelatin from Domestic Chicken Feet (*Gallus gallus domesticus*) with Variations in Soaking Time in $Ca(OH)_2$ and Citric Acid

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ABSTRACT

Gelatin is a hydrolysis product of collagen widely used in the food and pharmaceutical industries. Commercial gelatin is generally derived from cows and pigs, necessitating alternative halal gelatin sources, one of which is domestic chicken feet (*Gallus gallus domesticus*). This study aimed to determine the effect of variations in soaking time on the physical quality of gelatin from domestic chicken feet using the hydrolysis method with 0.2% calcium hydroxide ($Ca(OH)_2$) and 5% citric acid solution. The study employed an experimental method with a Completely Randomized Design (CRD) consisting of three soaking time treatments (4, 6, and 8 days) with three replications. Parameters tested included yield, organoleptic properties, homogeneity, pH, protein content, ash content, water content, and viscosity. Data were analyzed using the Shapiro-Wilk test, homogeneity test, One Way ANOVA, and Post Hoc test. The results showed that all gelatin samples had good physical quality. The pH values ranged from 5.20–5.69, ash content 2.37–2.55%, water content 3.37–3.83%, and viscosity 3.54–4.77 cP. The highest yield was obtained at 4 days of soaking (13.30%), while the lowest was at 8 days (4.80%). Statistical analysis showed that soaking time significantly affected yield, pH, and viscosity ($p<0.05$), but did not significantly affect ash content and water content ($p>0.05$). It was concluded that 4 days of soaking produced gelatin with the most optimal quality and yield.

Keyword: Domestic Chicken Feet, Gelatin, Hydrolisys, Physical Quality, Soaking Time

ABSTRAK

Gelatin merupakan hasil hidrolisis kolagen yang banyak dimanfaatkan dalam industri pangan dan farmasi. Gelatin komersial umumnya berasal dari sapi dan babi sehingga diperlukan alternatif sumber gelatin halal, salah satunya dari ceker ayam kampung (*Gallus gallus domesticus*). Penelitian ini bertujuan untuk mengetahui pengaruh variasi lama perendaman terhadap mutu fisik gelatin dari ceker ayam kampung menggunakan metode hidrolisis dengan larutan kalsium hidroksida ($Ca(OH)_2$) 0,2% dan asam sitrat 5%. Penelitian menggunakan metode eksperimental dengan Rancangan Acak Lengkap (RAL) yang terdiri dari tiga perlakuan lama perendaman yaitu 4 hari, 6 hari, dan 8 hari dengan tiga kali replikasi. Parameter yang diuji meliputi rendemen, organoleptik, homogenitas, pH, protein, kadar abu, kadar air, dan viskositas. Analisis data dilakukan menggunakan uji Shapiro-Wilk, uji homogenitas, One Way ANOVA, dan Post Hoc. Hasil penelitian menunjukkan bahwa seluruh sampel gelatin memiliki mutu fisik yang baik. Nilai pH berkisar 5,20–5,69, kadar abu 2,37–2,55%, kadar air 3,37–3,83%, dan viskositas 3,54–4,77 cP. Rendemen tertinggi diperoleh pada perendaman 4 hari sebesar 13,30%, sedangkan terendah pada 8 hari sebesar 4,80%. Analisis statistik menunjukkan bahwa lama perendaman berpengaruh signifikan terhadap rendemen, pH, dan viskositas ($p<0,05$), namun tidak berpengaruh signifikan terhadap kadar air dan kadar abu ($p>0,05$). Disimpulkan bahwa perendaman selama 4 hari menghasilkan gelatin dengan kualitas dan rendemen paling optimal.

Kata Kunci: Ceker Ayam Kampung, Gelatin, Hidrolisis, Lama Perendaman, Mutu Fisik

1. Introduction

Gelatin is a biopolymer resulting from the hydrolysis of collagen that functions as a thickening agent or texture former in various food products such as candy, yogurt, and bakery products [1]. Commercial gelatin available in the market generally originates from bovine bones and porcine skin. In Indonesia, this raises halal concerns as the majority of the population is Muslim. Therefore, efforts to find alternative halal gelatin sources continue to be pursued [2]. One potential alternative source of halal gelatin comes from chicken, particularly the feet part [3]. Chicken feet consist of skin, bones, muscles, and connective tissue, and are rich in collagen at approximately 9.07%. However, chicken feet are often considered waste or by-products of slaughter that are rarely utilized [4].

The hydrolysis method is a technique for extracting gelatin from collagen using acid or base treatment [5]. Various studies have shown that different types of acid and base solutions and soaking time affect the yield and physical quality of the resulting gelatin [6]. Research conducted by Hido et al. [7] using the acid method with acetic acid solution and extraction at 70°C for 5 hours successfully extracted 13.60% gelatin from domestic chicken feet. Meanwhile, research by Jannah et al. [8] using the hydrolysis method with a combination of 0.2% NaOH base solution and 5% citric acid at room temperature (28–30°C) for 8, 10, and 12 days successfully extracted gelatin from whole broiler chicken feet, with the best results at 8 days treatment, producing gelatin with optimum physical quality (pH 4.73; water content 9.71%; ash content 2.15%; and positive protein test). Research by Arifin et al. [9] using the hydrolysis method combining $Ca(OH)_2$ base solution and CH_3COOH acid solution at 60°C for 24 hours successfully extracted 25.14 ± 1.19% gelatin from dried chicken feet skin. Mokoolang et al. [10] using the base hydrolysis method with various concentrations of calcium hydroxide (15%, 20%, 25%, and 30%) without acid solution at room temperature for 4 days successfully extracted 10.82–11.35% gelatin from bovine skin.

Research specifically examining the variation of citric acid and potassium hydroxide use with varying soaking times in the hydrolysis process of domestic chicken feet (*Gallus gallus domesticus*) has not been extensively conducted [11]. The novelty of this study lies in the use of $Ca(OH)_2$ as a base solution combined with citric acid, which is more environmentally friendly compared to the commonly used KOH or NaOH, and the optimization of soaking time to produce gelatin with good physical quality. Based on this background, this study was conducted to determine the manufacturing process and the effect of the hydrolysis process combining 0.2% $Ca(OH)_2$ base solution and 5% citric acid at room temperature (28–30°C) with variations in soaking time of 4, 6, and 8 days on the physical quality of gelatin from domestic chicken feet (*Gallus gallus domesticus*).

2. Methods

2.1. Research Design

This research was an experimental study using a Completely Randomized Design (CRD) with three soaking time treatments (4, 6, and 8 days) and three replications for each parameter [12]. The treatment durations of 4, 6, and 8 days were selected based on preliminary experiments, which showed that soaking beyond 8 days caused excessive collagen degradation, resulting in a drastic yield decrease to <3% and poor physical quality. Therefore, 8 days was set as the upper limit to ensure measurable and meaningful data. The research was conducted at the Integrated Laboratory of Campus 3, Poltekkes Kemenkes Surakarta from September 2025 to April 2026.

2.2. Materials and Equipment

The materials used in this study included domestic chicken feet (*Gallus gallus domesticus*), 0.2% calcium hydroxide ($Ca(OH)_2$) solution (technical grade), 5% citric acid (technical grade), distilled water, and ninhydrin solution (pro analysis, Merck). The equipment used included aluminum foil, a 100 mesh sieve, a small basin, a stirring rod, a Miyako blender, a 1000 mL Pyrex beaker glass, a 100 mL Pyrex measuring cup, an oven tray, a water bath, an oven, a pH meter (Hanna Instruments), a knife, a cloth filter, a cutting board, crucible tongs, a porcelain crucible, test tubes, a dropper pipette, a furnace (Nabertherm), a digital balance (Ohaus), glass jars, a Brookfield viscometer (NDJ-85), and a water bath (EQUITRON 6824DI).

2.3. Gelatin Preparation Procedure

A total of 250 g of chicken feet were cleaned, washed, and soaked in warm water at 60°C for 30 minutes, then drained. The samples were soaked in 0.2% $Ca(OH)_2$ solution according to the treatment variations (4, 6, and 8 days) at room temperature (28–30°C). After soaking, the samples were rinsed with distilled water until neutral pH was achieved. Subsequently, the neutralized samples were soaked in 5% citric acid solution for 24

hours at room temperature (28–30°C). The extraction process was carried out using a magnetic stirrer hot plate at a constant temperature of 70°C for one hour with the addition of distilled water at a sample-to-solvent ratio of 1:3 (w/v) (i.e., 250 g of chicken feet samples were extracted with 750 mL of distilled water). This temperature was chosen based on literature optimization [7] and preliminary trials, as it effectively extracts gelatin while minimizing excessive degradation of the polypeptide chains, which can occur at higher temperatures (>80°C). The extract was filtered using cloth filters, and the filtrate was collected. The extract was then concentrated using a water bath at 70°C and dried in an oven at 90°C. The dried gelatin sheets were ground into powder using a blender before further testing [8].

2.4 Physical Quality Testing

The physical quality tests conducted included organoleptic, homogeneity, pH, protein, ash content, water content, and viscosity tests. Organoleptic test with observation of shape, color, aroma, and texture using human senses [13]. In homogeneity test the gelatin was applied to a glass slide and observed for uniform distribution without coarse particles [14]. The pH value was measured by dissolving 0.2 g of gelatin powder in 20 mL of distilled water, stirring the solution, and measuring it using a calibrated pH meter at room temperature [15]. Protein identification was conducted using the ninhydrin method, where 3 mL of gelatin sample solution was added with 1 drop of 0.1% ninhydrin solution, heated to boiling, and observed for color change to purple, indicating the presence of protein [16]. The ash content was determined by placing 1 g of sample in a furnace at 450–600°C for 4 hours, cooling it, and weighing the residue to calculate the mineral content [17]. Water content was measured using a moisture analyzer at approximately 105°C to determine the percentage of water in the sample [18]. Finally, viscosity testing was performed by preparing a 6.67% (w/w) gelatin solution (7 g of gelatin in 105 mL of distilled water) and measuring it using a Brookfield viscometer at 60°C with a spindle speed of 60 rpm [19]. All tests were conducted in triplicate to ensure accuracy and reproducibility of the results.

2.5. Data Analysis

Data were analyzed using Shapiro-Wilk normality test, homogeneity test, One Way ANOVA, and Post Hoc test at 95% confidence level [20]. If data were normally distributed and homogeneous, parametric One Way ANOVA was performed. If the significance value $p<0.05$, there was a significant difference between groups; if $p>0.05$, there was no significant difference. Non-normal and non-homogeneous data were analyzed using the Kruskal-Wallis test.

3. Results and Discussion

3.1. Yield

The yield of gelatin is an important parameter that determines the efficiency of the extraction process. It represents the percentage of gelatin obtained from the initial weight of the raw material. The yield of gelatin from domestic chicken feet at different soaking times is presented in Table 1. The gelatin produced in this study exhibits characteristics consistent with Type B gelatin (alkaline-processed gelatin), as we used calcium hydroxide as the base pretreatment. All measured parameters were compared against the SNI (Standar Nasional Indonesia) and GMIA (Gelatin Manufacturers Institute of America) standards.

Table 1. Yield of Gelatin from domestic chicken feet (*Gallus gallus domesticus*)

No	Soaking Time	Replication Yield			Mean ± SD	Standard
		R1	R2	R3		
1	4 Days	13.48%	13.18%	13.25%	$13.30^{a} \pm 0.15$	9.07% [7]
2	6 Days	9.11%	8.98%	8.99%	$9.02^{b} \pm 0.07$	
3	8 Days	5.10%	4.56%	4.76%	$4.80^{c} \pm 0.27$	

Note: Different superscripts indicate significant differences ($p < 0.05$) based on One Way ANOVA followed by Post Hoc test.

Based on the results, the yield decreased with increasing soaking time. The highest yield was obtained at 4 days (13.30%), while the lowest was at 8 days (4.80%). This decrease was attributed to excessive collagen degradation due to prolonged soaking, causing some collagen components to dissolve and be lost during washing [7]. In this study, the soaking time was limited to 8 days because preliminary trials indicated that extended soaking (>8 days) led to over-hydrolysis of collagen, significantly reducing both yield and viscosity, making the gelatin unsuitable for further analysis. Statistical analysis showed a significance value of $p < 0.001$, indicating that soaking time significantly affected the yield.

3.2. Organoleptic Test

The organoleptic test is a qualitative evaluation that provides initial information about the physical characteristics of the gelatin produced. The assessment includes observations of shape, color, and odor, which are important indicators of gelatin quality and consumer acceptance. The organoleptic test results of gelatin from domestic chicken feet are shown in Table 2.

Table 2. Organoleptic test results of gelatin from domestic chicken feet

Soaking Time	Shape	Color	Odor
4 Days	Powder	Yellowish***	Gelatin Characteristic
6 Days	Powder	Yellowish**	Gelatin Characteristic
8 Days	Powder	Yellowish*	Gelatin Characteristic

Note: Fewer asterisks (*) indicate lighter color

All samples produced powder-form gelatin with yellowish color and characteristic odor. The yellowish color indicated that the extraction and purification process had proceeded well [13]. The characteristic odor was attributed to interactions between amino acids and volatile compounds formed during processing [21].

3.3. Homogeneity Test

The homogeneity test is conducted to ensure that the gelatin powder has a uniform composition without any coarse particles or lumps that could affect the quality of the final product. This test is particularly important for gelatin applications in food and pharmaceutical industries where consistency is required. The homogeneity test results are presented in Table 3.

Table 3. Homogeneity test results of gelatin from domestic chicken feet

Soaking Time	Replication		
	R1	R2	R3
4 Days	Homogeneous	Homogeneous	Homogeneous
6 Days	Homogeneous	Homogeneous	Homogeneous
8 Days	Homogeneous	Homogeneous	Homogeneous

All samples showed homogeneous properties, indicated by the absence of coarse particles or lumps [14]. This confirmed that the extraction and grinding processes were optimal.

3.4. pH Test

The pH value is an important quality parameter that affects the functional properties of gelatin, including its viscosity, gel strength, and stability. The pH of gelatin is influenced by the extraction method and the type of acid or base used during the hydrolysis process. The pH test results of gelatin from domestic chicken feet are shown in Table 4.

Table 4. pH test results of gelatin from domestic chicken feet

Soaking Time	Replication			Mean ± SD	Standard
	R1	R2	R3		
4 Days	5.68	5.66	5.69	$5.67^{a} \pm 0.01$	4.5 – 6.5
6 Days	5.34	5.30	5.35	$5.33^{b} \pm 0.02$	[22]
8 Days	5.20	5.21	5.23	$5.21^{c} \pm 0.01$	

Note: Different superscripts indicate significant differences ($p < 0.05$) based on One Way ANOVA followed by Post Hoc test.

The pH values ranged from 5.20–5.69, all within the SNI standard (4.5–6.5). The pH decreased with longer soaking time due to increased penetration of H^+ ions into the collagen matrix [22]. Statistical analysis showed significant differences between all treatment groups ($p < 0.05$).

3.5. Protein Test

The protein test is performed to confirm the presence of free amino groups in the gelatin sample. The ninhydrin test is a specific qualitative test for α-amino acids, where a positive result is indicated by the formation of a purple or violet color. The protein test results of gelatin from domestic chicken feet are presented in Table 5.

Table 5. Protein test results of gelatin from domestic chicken feet

Soaking Time	Replication			Standard
	R1	R2	R3	
4 Days	Purple	Purple	Purple	Purple
6 Days	Purple	Purple	Purple	[16]
8 Days	Purple	Purple	Purple	

All samples showed positive results for protein content, indicated by the formation of purple color with ninhydrin reagent. This confirmed the presence of free amino groups in the gelatin produced [16]. This result is consistent with the study by Sari and Yuliastuti [25], which also confirmed the presence of protein in chicken feet gelatin using the ninhydrin test.

3.6. Ash Content Test

The ash content test determines the amount of mineral or inorganic matter remaining in the gelatin after complete combustion. The ash content is an indicator of the purity of gelatin, where lower ash content indicates higher purity. The ash content test results of gelatin from domestic chicken feet are shown in Table 6.

Table 6. Ash content test results of gelatin from domestic chicken feet

Soaking Time	Replication			Mean ± SD	Standard
	R1	R2	R3		
4 Days	2.38%	2.22%	2.52%	$2.37^{a} \pm 0.15$	$\leq 3.25\%$
6 Days	2.61%	2.46%	2.35%	$2.47^{a} \pm 0.13$	[23]
8 Days	2.57%	2.36%	2.72%	$2.55^{a} \pm 0.18$	

Note: Same superscripts indicate significant differences ($p > 0.05$) based on One Way ANOVA followed by Post Hoc test.

All ash content values remained below the SNI maximum limit of 3.25%. The ash content tended to increase with longer soaking time due to mineral residues from the soaking solutions being trapped in the loosening collagen matrix [23]. Statistical analysis showed no significant difference ($p > 0.05$).

3.7. Water Content Test

Water content is a critical quality parameter that affects the stability, shelf life, and handling characteristics of gelatin powder. Lower water content indicates better drying efficiency and reduced risk of microbial growth during storage. The water content test results of gelatin from domestic chicken feet are presented in Table 7.

Table 7. Water content test results of gelatin from domestic chicken feet

Soaking Time	Replication			Mean ± SD	Standard
	R1	R2	R3		
4 Days	3.30%	3.43%	3.37%	$3.37^{a} \pm 0.07$	$\leq 16\%$
6 Days	2.93%	3.75%	3.64%	$3.44^{a} \pm 0.45$	[23]
8 Days	3.61%	3.86%	4.02%	$3.83^{a} \pm 0.21$	

Note: Same superscripts indicate significant differences ($p > 0.05$) based on One Way ANOVA followed by Post Hoc test.

All water content values were far below the SNI maximum limit of 16%, indicating optimal drying process and stable product for storage [18]. The water content showed a tendency to increase with longer soaking time due to more open collagen structures that can bind more water [24]. Statistical analysis showed no significant difference ($p > 0.05$).

3.8. Viscosity Test

Viscosity is an important rheological property that reflects the ability of gelatin to form a gel and bind water. Higher viscosity indicates the presence of longer polypeptide chains and better gel-forming properties. The viscosity of gelatin is influenced by the extraction conditions, including soaking time and temperature. The viscosity test results of gelatin from domestic chicken feet are shown in Table 8.

Table 8. Viscosity test results of gelatin from domestic chicken feet

Soaking Time	Replication			Mean ± SD	Standard
	R1	R2	R3		

4 Days	4.8	4.2	5.3	$4.77^{a} \pm 0.55$	$1.5 - 7.5$
6 Days	3.9	4.1	4.0	$4.00^{ab} \pm 0.10$	cP
8 Days	3.3	3.5	3.8	$3.54^{b} \pm 0.25$	[19]

Note: Different superscripts indicate significant differences ($p < 0.05$) based on One Way ANOVA followed by Post Hoc test. The 4-day treatment (^{a}) was significantly different from the 8-day treatment (^{b}), while the 6-day treatment (^{ab}) was not significantly different from either.

The viscosity values ranged from 3.54–4.77 cP, all within the GMIA standard (1.5–7.5 cP). Viscosity decreased with longer soaking time because the collagen polypeptide chains were broken into shorter molecules, reducing the water-binding capacity and thickness [7]. Statistical analysis showed significant differences between 4-day and 8-day treatments ($p < 0.05$), while the 6-day treatment was not significantly different from either the 4-day or 8-day treatments.

4. Conclusion

Based on the research results, the following conclusions can be drawn: variations in soaking time using 0.2% calcium hydroxide and 5% citric acid solution affected the yield, pH, and viscosity of gelatin from domestic chicken feet (*Gallus gallus domesticus*), but did not significantly affect ash content and water content. All gelatin samples produced showed good organoleptic characteristics and homogeneity, meeting gelatin quality standards based on pH, ash content, water content, and viscosity parameters. The 4-day soaking treatment was the most optimal treatment, producing the highest yield (13.30%) and best viscosity (4.77 cP) compared to the 6-day and 8-day treatments. Longer soaking time caused a decrease in yield and viscosity due to excessive collagen degradation during the hydrolysis process. This research has limitations, including the absence of FT-IR spectroscopy analysis to confirm the functional groups of the gelatin and the lack of gel strength measurement. This study evaluated physical quality parameters including yield, organoleptic properties, homogeneity, pH, protein content, ash content, water content, and viscosity. Gel strength was not measured in this study, and therefore claims regarding gel-forming performance are limited to the parameters tested. Future studies are recommended to include FT-IR spectroscopy and gel strength analysis to provide a more complete characterization of the gelatin's functional groups and gel-forming performance.

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