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# Comparative Effect of Acetic Acid and Bilimbi (*Averrhoa bilimbi* L.) Extract on the Extraction and Characteristics of Chicken Skin Gelatin

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**Abstract:** Chicken skin is a potential halal collagen source for gelatin production, especially as an alternative to bovine and porcine gelatin. This study aimed to compare acetic acid and bilimbi fruit extract (*Averrhoa bilimbi* L.) as acid pretreatment agents for extracting gelatin from chicken skin. The novelty of this study lies in comparing a common food-grade acid with a local natural acid source for sustainable halal gelatin production from poultry by-products. Chicken skin was pretreated with 0.15% NaOH, soaked in acetic acid or bilimbi extract at 2%, 3%, and 4% (m/v) for 24 h, extracted at 65°C for 90 min, and dried at 55°C for 24 h. The gelatin was analyzed for yield, moisture content, ash content, pH, and Fourier Transform Infrared (FTIR) spectra. Data were presented as mean ± standard deviation and analyzed using two-way ANOVA followed by Tukey's HSD test at $p < 0.05$. The results showed that gelatin yield ranged from 2.35 ± 0.04% to 10.65 ± 0.07%. Acetic acid produced higher yield than bilimbi extract at the same concentration. The best treatment was 4% acetic acid, which produced the highest yield (10.65 ± 0.07%), low moisture content (9.42 ± 0.08%), low ash content (1.48 ± 0.04%), and pH of 4.48 ± 0.02. FTIR analysis showed amide A, amide B, amide II, and amide III bands, indicating partial hydrolysis of collagen into gelatin. Although bilimbi extract showed lower extraction efficiency, it still has potential as a sustainable local acid source for halal gelatin extraction. These findings support the utilization of chicken skin and local natural resources for sustainable halal gelatin production.

**Keywords:** Acetic Acid; Bilimbi Fruit Extract; Chicken Skin Gelatin; FTIR Analysis; Halal Gelatin.

## Introduction

Gelatin is a protein obtained from the partial hydrolysis of collagen present in animal connective tissues such as skin, bones, tendons, and cartilage. Due to its unique functional properties, including gel-forming ability, emulsification, water-binding capacity, and viscosity enhancement, gelatin has been widely utilized in food, pharmaceutical, cosmetic, and biomedical industries [1].

Commercial gelatin is predominantly produced from bovine and porcine sources. However, porcine-derived gelatin is not acceptable for Muslim consumers because of halal restrictions, while bovine-derived gelatin may raise concerns related to disease transmission, religious acceptability, and consumer preference [2]. Therefore, the development of alternative halal gelatin sources has become increasingly important, particularly in countries with large Muslim populations such as Indonesia.

Poultry by-products, especially chicken skin, represent a promising alternative source of halal gelatin because they contain considerable amounts of collagen and are abundantly generated as waste from the poultry processing industry [3]. The utilization of chicken skin for gelatin production not only provides added economic value to poultry waste but also contributes to sustainable waste management and the development of halal biomaterials. Previous studies have shown that gelatin extracted from poultry by-products has potential physicochemical and functional properties for industrial applications, although its quality is strongly influenced by raw material characteristics and extraction conditions [4].

The extraction of gelatin generally involves acid or alkaline pretreatment to disrupt the collagen triple-helix structure and facilitate its conversion into gelatin during thermal extraction. Acid pretreatment is particularly effective for less cross-linked collagen materials because it promotes collagen swelling, weakens intermolecular cross-links, and increases collagen solubility [1]. Acid pretreatment helps weaken the collagen structure by allowing acid to enter the collagen fibers. This process can weaken hydrogen bonds and other intermolecular interactions in collagen, making the triple-helix structure more susceptible to disruption during heating. As a result, collagen can be partially hydrolyzed and converted into gelatin more easily [5]. However, the effectiveness of acid pretreatment is determined not only by the acidity level but also by the acid type, acid strength, molecular size, diffusion ability, and the presence of other compounds in the pretreatment medium. Therefore, differences in acid type and concentration may result in different collagen hydrolysis efficiencies, gelatin yields, and physicochemical properties [6].

Among various food-grade acids, acetic acid is one of the most commonly used in gelatin extraction due to its effectiveness, safety, and relatively stable hydrolysis. Previous studies reported that acetic acid treatment successfully enhanced gelatin extraction from chicken by-products [5]. Acetic acid is a purified organic acid with a

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more stable composition than natural acid extracts. This condition allows acetic acid to act more consistently in swelling collagen fibers and increasing collagen solubility during gelatin extraction. However, its use still needs to be controlled properly, especially during washing, to reduce acid residues and maintain the quality of the gelatin produced [7].

Recently, growing attention has been directed toward the utilization of natural acid sources as environmentally friendly alternatives in gelatin extraction processes. One potential natural acid source is bilimbi fruit (*Averrhoa bilimbi* L.), a tropical fruit characterized by its strong acidic taste due to the presence of various organic acids, particularly oxalic acid and ascorbic acid [8]. Bilimbi fruit extract has been successfully applied as a natural acidifying and coagulating agent in food processing applications [9]. Bilimbi extract was used in this study because its organic acid content may assist collagen swelling and partial hydrolysis during pretreatment. Moreover, bilimbi is locally available, low-cost, and has potential as a natural acid source for halal gelatin extraction. Bilimbi extract may produce different effects from acetic acid because it is a natural extract with a more complex composition. Besides organic acids, it also contains minerals, sugars, and other soluble compounds. These compounds may affect collagen swelling, solubility, ash content, and water retention in the extracted gelatin. Therefore, lower pH does not always result in higher gelatin yield or better gelatin quality.

Several recent studies have explored the use of natural acids to extract gelatin from various animal sources. Azrita et al. (2025) reported that *Averrhoa bilimbi* extract could be utilized as a pretreatment agent for fish scale gelatin extraction, producing acceptable functional properties [10]. In addition, Rustan and Andriani (2025) investigated broiler chicken intestine gelatin extraction using bilimbi extract and obtained moderate gelatin yield values [11]. However, these previous studies were conducted on different raw materials, such as fish scales and chicken intestine, which have different collagen structures, crosslink densities, fat contents, and mineral compositions compared with chicken skin. Previous studies have mostly focused on the use of natural acids alone, while direct comparisons with common food-grade acids under the same extraction conditions remain limited. Thus, the effectiveness of bilimbi extract compared with acetic acid for extracting chicken skin gelatin requires further investigation.

In addition, information on how acid type and concentration affect the yield, physicochemical properties, and functional groups of chicken skin gelatin is still limited. Bhargavi et al. (2025) emphasized that extraction conditions, acid treatment, and pretreatment methods strongly affect the quality and functionality of poultry-derived gelatin [12].

Therefore, this study aimed to compare the effects of acetic acid and bilimbi fruit extract at different concentrations on the extraction yield and physicochemical characteristics of chicken skin gelatin. The novelty of this study lies in the direct comparison between a conventional food-grade acid and a locally available natural acid source for halal gelatin extraction from chicken skin. In addition, this study evaluates whether bilimbi extract can serve as a natural acid source to promote collagen hydrolysis and gelatin formation, compared with acetic acid, a commonly used food-grade acid. The findings of this study are expected to contribute to the development of sustainable halal gelatin production utilizing poultry by-products and local natural resources.

## Research Methods

### Materials

Fresh chicken skin used in this study was obtained from a local poultry market in Padang. Bilimbi fruits (*Averrhoa bilimbi* L.) were collected from local plantations. The chemicals used included sodium hydroxide (NaOH), acetic acid ($CH_3COOH$), and distilled water. All chemicals used in this study were of analytical grade.

### Experimental Design

This study employed a laboratory experimental method with a factorial design comprising two factors: acid type and acid concentration. The acid types were acetic acid and bilimbi fruit extract, and the acid concentrations applied were 2%, 3%, and 4% (m/v). The treatment codes used in this study are presented in Table 1. All treatments were carried out in triplicate, and the data were expressed as mean ± standard deviation.

**Table 1.** Treatment codes for acid type and concentration used in gelatin extraction

| Code | Acid Type | Conc. |
|---|---|---|
| A1 | Acetic acid | 2% |
| A2 | Acetic acid | 3% |
| A3 | Acetic acid | 4% |
| B1 | Bilimbi fruit extract | 2% |
| B2 | Bilimbi fruit extract | 3% |
| B3 | Bilimbi fruit extract | 4% |

### Equipment

The equipment used in this study included beakers, volumetric flasks, measuring cylinders, analytical balance (ABS 220-4), blender, filter paper, hot plate, thermometer, dehydrator, pH meter, spatula, glass rods, water bath (B-One DWB-1H-3L), and Fourier Transform Infrared (FTIR) spectrophotometer.

### Preparation of Chicken Skin

Fresh chicken skin was washed thoroughly under running water to remove adhering fat, dirt, and residual meat. The cleaned chicken skin was then cut into small pieces approximately 2–3 cm in size. Subsequently, the samples were dried using a dehydrator at 40°C for 40 min, following the method reported by Işık et al. (2024) with slight modifications [13].

### Preparation of Bilimbi Fruit Extract

Fresh bilimbi fruits (*Averrhoa bilimbi* L.) weighing approximately 1 kg were washed and cut into small pieces. The fruits were blended without water to obtain a homogeneous slurry. The slurry was filtered using filter paper to separate the filtrate from the solid residue. The resulting filtrate was used as crude bilimbi fruit extract for the acid pretreatment process [14]. Bilimbi extract concentrations of 2%, 3%, and 4% (v/v) were prepared by adding 2 g, 3 g, and 4 g of crude bilimbi extract, respectively, and diluting each to a final volume of 100 mL with distilled water.

**Alkaline Pretreatment**

A total of 15 g of dried chicken skin was soaked in 200 mL of 0.15% NaOH solution (m/v) and stirred for 40 min. The soaking process was repeated three times, with the fresh NaOH solution replaced every 40 min. After alkaline treatment, the samples were washed using distilled water until a neutral pH (6–7) was achieved to remove residual alkali, fat, and impurities [15].

**Acid Soaking Process**

The pretreated chicken skin samples were soaked in either acetic acid solution or bilimbi fruit extract at concentrations of 2%, 3%, and 4% (m/v). The soaking process was carried out for 24 h at room temperature. The 24 h soaking time was used to allow the acid solution to penetrate the chicken skin and help swell the collagen fibers before extraction [16]. This soaking time was considered sufficient for the acid solution to interact with the collagen fibers. After acid pretreatment, the samples were washed with distilled water until neutral pH was reached.

**Gelatin Extraction**

Gelatin extraction was performed using a thermal extraction method. The acid-treated chicken skin samples were heated in a water bath at 65°C for 90 min. The extraction was carried out at 65°C because this temperature can support collagen conversion into gelatin without causing excessive protein degradation [17]. The extraction time was set to 90 min to allow sufficient heating to convert collagen into gelatin. This condition was adapted from previous gelatin extraction methods with slight modifications [18]. The extracted solution was filtered using filter paper to separate the liquid gelatin extract from the remaining solid residue.

**Drying Process**

The gelatin filtrate obtained from the extraction process was dried in a dehydrator at 55°C for 24 h, forming dry gelatin sheets. The dried gelatin was then ground into powder and stored in airtight containers prior to further analysis.

**Gelatin Characterization**

*Gelatin Yield*

Gelatin yield was calculated based on the ratio of dry gelatin weight to the initial dry weight of chicken skin used for extraction and expressed as a percentage (%).

$$\text{Yield (\%)} = \frac{\text{Weight of dry gelatin}}{\text{Initial weight of dried chicken skin}} \times 100$$

*Moisture Content*

Moisture content analysis was conducted according to the AOAC method [19]. Approximately 1 g of gelatin sample was dried in an oven at 100°C for 6 h until constant weight was obtained. Moisture content was calculated from the difference between the initial and final sample weights.

*Ash Content*

Ash content analysis was performed following the AOAC method [19]. Approximately 1 g of gelatin sample was incinerated in a furnace at 500°C until complete ash formation. The ash content was expressed as a percentage (%) based on the remaining inorganic residue relative to the initial sample weight.

*pH Measurement*

The pH value of gelatin was determined according to the method described by Ozyurt et al. 2017 [20]. A 1% (m/v) gelatin solution was prepared by dissolving gelatin in distilled water at 55–60°C with continuous stirring for 30 min. The solution was cooled to room temperature before measuring its pH with a calibrated pH meter.

*FTIR Analysis*

Fourier Transform Infrared (FTIR) analysis was conducted to identify the functional groups present in the extracted gelatin. FTIR characterization was performed on the best treatment sample (A3), selected based on the highest gelatin yield, lowest moisture content, and lowest ash content. Spectral analysis was carried out within the wavenumber range of 4000–400 $cm^{-1}$ using an FTIR spectrophotometer.

*Statistical Analysis*

Data were expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using IBM SPSS Statistics software. The data were analyzed using two-way analysis of variance (ANOVA) to determine the effects of acid type, acid concentration, and their interaction on gelatin yield, moisture content, ash content, and pH. When significant differences were found at $p < 0.05$, Tukey's Honestly Significant Difference (HSD) test was used as a post hoc test.

## Results and Discussion

**Gelatin Yield**

Gelatin yield is one of the most important parameters for evaluating the effectiveness of collagen conversion during extraction. The yield of chicken skin gelatin obtained in this study ranged from 2.35 ± 0.04% to 10.65 ± 0.07%, as presented in Table 2. In general, acetic acid treatment produced higher gelatin yield than bilimbi fruit extract at the same concentration. Two-way ANOVA showed that acid type, concentration, and their interaction significantly affected gelatin yield ($p < 0.001$). Tukey's HSD test showed that all treatments were significantly different ($p < 0.05$).

**Table 2.** Yield of chicken skin gelatin extracted using acetic acid and bilimbi fruit extract

| Code | Acid Type | Conc. | Yield (%) |
|---|---|---|---|
| A1 | Acetic acid | 2% | 7.50 ± 0.05$^{c}$ |
| A2 | Acetic acid | 3% | 10.40 ± 0.08$^{b}$ |
| A3 | Acetic acid | 4% | 10.65 ± 0.07$^{a}$ |
| B1 | Bilimbi fruit extract | 2% | 2.35 ± 0.04$^{f}$ |
| B2 | Bilimbi fruit extract | 3% | 2.95 ± 0.05$^{e}$ |
| B3 | Bilimbi fruit extract | 4% | 3.65 ± 0.06$^{d}$ |

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The highest yield was obtained from treatment A3, while the lowest was from treatment B1. This result shows that acetic acid was more effective than bilimbi extract in helping collagen swelling and gelatin formation during extraction. Acid pretreatment can weaken the collagen structure by allowing acid to enter the collagen fibers [21]. This process reduces hydrogen bonds and intermolecular interactions, so the triple-helix structure becomes easier to break down during heating and can be converted into gelatin [5].

Increasing acetic acid concentration improved gelatin yield, particularly from 2% to 3%. However, the increase from 3% to 4% was relatively small, suggesting that collagen solubilization may have nearly reached its optimum. Similar results were reported by Kumar et al. (2016), who found that higher acetic acid concentration improved gelatin extraction from chicken feet [22].

The yield from bilimbi extract treatments also increased with increasing concentration. This indicates that the organic acids in bilimbi extract still played a role in swelling and hydrolyzing collagen [23]. However, the yield was lower than that of acetic acid treatments. This result shows that acidity alone did not determine gelatin yield. Although bilimbi extract contains organic acids, it is not a pure acid solution and has a more complex composition than acetic acid.

The lower yield of bilimbi extract may be due to other compounds present in the extract, such as minerals, sugars, and phytochemicals. These compounds may affect the ability of the acid to enter collagen fibers and reduce collagen swelling and solubility during heating. In addition, the acidity of bilimbi extract may not always be the same because it can be influenced by fruit maturity, water content, and extraction conditions [24]. Therefore, bilimbi extract may give less consistent results than acetic acid, which has a more stable composition. This also shows that a lower pH or a more acidic taste does not always result in a higher gelatin yield.

These results show that acid type affected gelatin yield. At the same concentration, acetic acid produced higher yield than bilimbi extract. This may be because acetic acid has a more stable composition, while the effectiveness of bilimbi extract can be influenced by the raw material and extraction conditions. Rustan and Andriani (2025) also reported low gelatin yield from broiler chicken intestine extracted using bilimbi fruit extract [11]. In addition, Azrita et al. (2025) stated that the effectiveness of *Averrhoa bilimbi* extract as a pretreatment agent depends on the type of raw material and extraction conditions [10].

Based on the yield results, gelatin yield increased with increasing acid concentration, but the effect was still influenced by the type of acid used. Acetic acid gave a better yield than bilimbi extract, especially at 4% concentration. Although the yield from bilimbi extract was lower, it still has potential as a natural acid source for halal gelatin extraction. Further improvement is needed by controlling factors such as extract acidity, fruit maturity, soaking time, and extraction conditions.

**Physicochemical Characteristics of Gelatin**

*Moisture Content*

Moisture content is an important parameter in determining gelatin quality because it affects storage stability, shelf life, and susceptibility to microbial deterioration. The moisture content of chicken skin gelatin obtained in this study ranged from 9.42 ± 0.08% to 11.86 ± 0.14%, as presented in Table 3. Statistical analysis showed that moisture content was significantly different among treatments ($p < 0.001$). Tukey's HSD test also showed significant differences among all treatment groups ($p < 0.05$).

**Table 3.** Moisture content of chicken skin gelatin extracted using acetic acid and bilimbi fruit extract

| Code | Acid Type | Conc. | Moisture Content (%) |
|---|---|---|---|
| A1 | Acetic acid | 2% | 10.35 ± 0.12$^{c}$ |
| A2 | Acetic acid | 3% | 9.84 ± 0.10$^{b}$ |
| A3 | Acetic acid | 4% | 9.42 ± 0.08$^{a}$ |
| B1 | Bilimbi fruit extract | 2% | 10.76 ± 0.11$^{d}$ |
| B2 | Bilimbi fruit extract | 3% | 11.28 ± 0.14$^{e}$ |
| B3 | Bilimbi fruit extract | 4% | 11.86 ± 0.14$^{f}$ |

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The moisture content of gelatin extracted with acetic acid decreased as the acid concentration increased. Treatment A3 showed the lowest moisture content, indicating that water was removed more effectively during drying. This may occur because higher acetic acid concentration helped break down the collagen structure more effectively, so less water was retained in the gelatin matrix.

In contrast, the moisture content of gelatin treated with bilimbi extract increased with increasing extract concentration. This may be due to the complex composition of the bilimbi extract. Besides organic acids, bilimbi extract also contains sugars, minerals, and other soluble compounds that can retain water in the gelatin matrix. As a result, water removal during drying may become less effective.

The differences in moisture content between acetic acid and bilimbi extract treatments indicate that the type of acid affected water retention in the gelatin. Acetic acid treatment produced gelatin with lower moisture content, while bilimbi extract treatment tended to retain more water. This may be due to soluble compounds in the bilimbi extract that bind water within the gelatin matrix. Sasmitaloka and Budiyanto (2022) also stated that strong interactions between gelatin molecules and water can reduce water evaporation during drying [25].

All moisture content values in this study were still below the maximum limit of SNI 06-3735-1995, which is 16%. This means that all gelatin samples met the Indonesian standard for moisture content. However, gelatin treated with acetic acid had lower moisture content, which may be better for storage because lower water content can reduce microbial growth and product damage.

*Ash Content*

Ash content represents the amount of inorganic minerals remaining in gelatin after incineration and is commonly used as an indicator of mineral residue and purification efficiency. The ash content values obtained in this study ranged from 1.48 ± 0.04% to 2.36 ± 0.05%, as shown in Table 4. Statistical analysis showed that ash content was significantly different among treatments ($p < 0.001$). Tukey's HSD test showed that most treatments were significantly different, except between A1 and B1.

**Table 4.** Ash content of chicken skin gelatin extracted using acetic acid and bilimbi fruit extract

| Code | Acid Type | Conc. | Ash Content (%) |
|---|---|---|---|
| A1 | Acetic acid | 2% | $1.85 \pm 0.04^{c}$ |
| A2 | Acetic acid | 3% | $1.62 \pm 0.04^{b}$ |
| A3 | Acetic acid | 4% | $1.48 \pm 0.04^{a}$ |
| B1 | Bilimbi fruit extract | 2% | $1.92 \pm 0.04^{c}$ |
| B2 | Bilimbi fruit extract | 3% | $2.14 \pm 0.05^{d}$ |
| B3 | Bilimbi fruit extract | 4% | $2.36 \pm 0.05^{e}$ |

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The ash content of gelatin treated with acetic acid decreased as the acid concentration increased. The lowest ash content was found in treatment A3. This result suggests that a higher acetic acid concentration helped remove mineral residues during soaking and washing. Acid treatment may release inorganic compounds from the collagen matrix, while washing helps reduce the remaining minerals in the gelatin [26]. Chand et al. (2021) also reported that alkaline and acid pretreatment reduced the ash content of chicken skin gelatin [27].

In contrast, gelatin treated with bilimbi fruit extract had higher ash content, especially at higher extract concentrations. This may be caused by the minerals and soluble inorganic compounds naturally present in bilimbi extract. When the extract concentration increased, more mineral components may have remained in the gelatin solution. Therefore, the ash content of gelatin treated with bilimbi extract was higher than that of gelatin treated with acetic acid.

This result shows that the type of acid used affected the mineral residue in gelatin. Acetic acid is a purified acid, so it did not add many mineral components during pretreatment. In comparison, bilimbi extract has a more complex composition because it is a natural extract. Besides organic acids, it may also contain minerals that can increase the gelatin's ash content. Therefore, the higher ash content in bilimbi-treated gelatin may be related to mineral residues from the extract [28].

High ash content is usually not expected, as it may indicate that the gelatin still contains residual minerals. Even so, all ash content values in this study were below the maximum limit specified in SNI 06-3735-1995, which is 3.25%. This means that all gelatin samples met the Indonesian standard for ash content. The lower ash content in acetic acid-treated gelatin also shows that acetic acid was more effective in reducing mineral residues than bilimbi fruit extract.

*pH Value*

pH values of chicken skin gelatin obtained in this study ranged from 3.92 ± 0.02 to 5.05 ± 0.02, as presented in Table 5. Statistical analysis showed that pH values were significantly different among treatments ($p < 0.001$). Tukey's HSD test showed that all treatments were significantly different ($p < 0.05$).

**Table 5.** pH values of chicken skin gelatin extracted using acetic acid and bilimbi fruit extract

| Code | Acid Type | Conc. | pH |
|---|---|---|---|
| A1 | Acetic acid | 2% | $5.05 \pm 0.02^{a}$ |
| A2 | Acetic acid | 3% | $4.76 \pm 0.02^{b}$ |
| A3 | Acetic acid | 4% | $4.48 \pm 0.02^{c}$ |
| B1 | Bilimbi fruit extract | 2% | $4.55 \pm 0.02^{d}$ |
| B2 | Bilimbi fruit extract | 3% | $4.18 \pm 0.02^{e}$ |
| B3 | Bilimbi fruit extract | 4% | $3.92 \pm 0.02^{f}$ |

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The pH value decreased as the acid concentration increased in both acetic acid and bilimbi extract treatments. This shows that higher acid concentration made the gelatin more acidic. During soaking, acid may enter the collagen fibers and some acid residues may remain after washing [29]. As a result, gelatin treated with higher acid concentration had lower pH values.

Gelatin treated with bilimbi fruit extract had lower pH values than gelatin treated with acetic acid at the same concentration. This may be due to the organic acids present in the bilimbi extract. As a natural extract, the acidity of bilimbi can also vary depending on fruit maturity, water content, and extract composition [30]. These conditions may make bilimbi-treated gelatin more acidic than acetic acid-treated gelatin.

The highest pH value was obtained in treatment A1, while the lowest pH value was obtained in treatment B3. This result shows that the pH of gelatin was affected by both acid type and acid concentration. A lower pH may affect gelatin solubility, stability, and gel formation [31]. Therefore, the washing step after acid soaking needs to be properly controlled to remove excess acid and obtain gelatin at a suitable pH.

Based on these results, gelatin treated with acetic acid had higher pH values than gelatin treated with bilimbi fruit extract. This shows that acetic acid treatment was easier to control during soaking and washing. Bilimbi fruit extract still has potential as a natural acid source, but its use needs better control, especially regarding extract concentration, acidity, and the washing process.

*FTIR Analysis*

FTIR analysis was conducted to identify the functional groups present in the extracted gelatin. The FTIR spectrum of gelatin extracted under the best treatment condition (A3) showed characteristic absorption bands at 3264 $cm^{-1}$, 2936 $cm^{-1}$, 1521 $cm^{-1}$, and 1242 $cm^{-1}$, as shown in Figure 1.

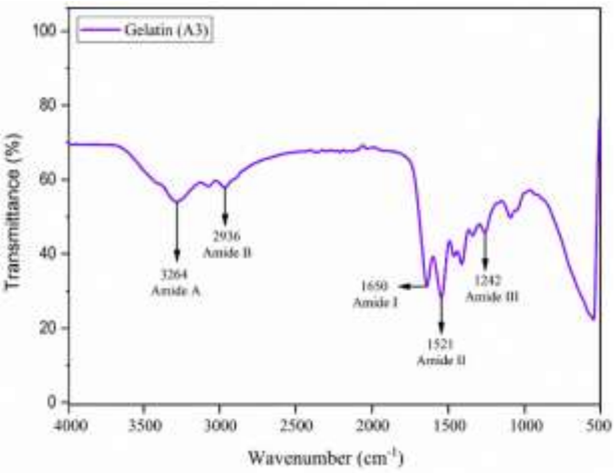


**Figure 1.** FTIR spectrum of chicken skin gelatin extracted under the best treatment condition (A3)

The FTIR spectrum obtained in this study showed absorption bands commonly observed in gelatin protein structures. The presence of amide bands indicated that the collagen triple-helix structure had been partially hydrolyzed into gelatin during thermal extraction. Similar spectral characteristics were reported in studies of poultry-derived gelatin, confirming successful extraction of gelatin from chicken skin.

The absorption band at 3264 $cm^{-1}$ was identified as amide A, which is related to N–H stretching and hydrogen bonding in gelatin [32]. This band shows the presence of peptide groups in the gelatin structure. The band at 2936 $cm^{-1}$ was assigned to amide B, which is associated with $CH_2$ stretching. This indicates the presence of aliphatic groups in the gelatin protein chain.

The absorption band at 1521 $cm^{-1}$ was identified as amide II, which is related to N–H bending and C–N stretching. Meanwhile, the band at 1242 $cm^{-1}$ was assigned to amide III, which is associated with C–N stretching and N–H deformation. The presence of these bands indicates that the extracted gelatin still retained the main protein functional groups.

These FTIR results show that treatment A3 produced gelatin with typical amide bands. The use of acetic acid in the pretreatment process may have helped loosen the collagen structure, while heating helped convert collagen into gelatin through partial hydrolysis [4]. Similar FTIR patterns have also been reported in previous studies on poultry gelatin, which showed typical protein functional groups after gelatin extraction [33].

Overall, the FTIR spectrum supports that gelatin was successfully extracted from chicken skin under the A3 treatment. The presence of amide A, amide B, amide II, and amide III shows that the extracted product had the main functional groups commonly found in gelatin. This result also supports the yield and physicochemical results, where A3 was selected as the best treatment in this study.

## Conclusion

The type and concentration of acid affected the yield and physicochemical characteristics of chicken skin gelatin. Acetic acid produced higher gelatin yield than bilimbi fruit extract at the same concentration. The best result was obtained from 4% acetic acid treatment (A3), which produced the highest yield (10.65 ± 0.07%), the lowest moisture content (9.42 ± 0.08%), and the lowest ash content (1.48 ± 0.04%). Meanwhile, the highest yield from bilimbi fruit extract was obtained at 4% concentration (B3), at 3.65 ± 0.06%. FTIR analysis revealed several functional groups commonly found in gelatin, including amide A, amide B, amide II, and amide III. The presence of these bands indicates that collagen from chicken skin was partially hydrolyzed into gelatin. Overall, acetic acid gave better extraction results and produced gelatin with better physicochemical characteristics than bilimbi fruit extract. Although bilimbi fruit extract yielded less, it still has potential as a natural local acid source for halal gelatin extraction. However, its use needs further optimization, especially in terms of extract acidity, fruit maturity, soaking time, washing process, and extraction conditions. Better control of these factors may help improve the yield and quality of gelatin produced using bilimbi fruit extract. This study also demonstrates that chicken skin can serve as a value-added raw material for halal gelatin production. The gelatin produced from chicken skin has potential applications in food, pharmaceutical, and biomaterial industries, depending on its quality and functional properties. Therefore, this study supports the development of sustainable halal biomaterials by utilizing poultry processing by-products and local natural resources.

**Author's Contribution**

M. Hurahmi contributed to the research conceptualization, sample preparation, gelatin extraction process, physicochemical characterization, data collection, data analysis, and manuscript drafting. M. Khair supervised the research design, provided methodological guidance, validated the analytical results, reviewed and revised the manuscript critically for important intellectual content, and approved the final version of the manuscript for publication.

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## References

[1] B. Bhernama, R. S. Nasution, and S. U. Nisa, "Ekstraksi Gelatin dari Tulang Ikan Kakap Putih ( Lates calcarifer ) Dengan Variasi Konsentrasi Asam HCl," *J. Sains Nat. Univ. Nusa Bangsa*, vol. 10, no. 2006, pp. 43–54, 2018, doi: https://doi.org/10.31938/jsn.v10i2.282.

[2] N. Aina, A. Anuar, N. A. Tukiran, M. A. Jamaludin,

and J. Gombak, "Gelatin in Halal Pharmaceutical Products," *Malaysian J. Syariah Law*, vol. 11, no. 1, pp. 64–78, 2023, doi: https://doi.org/10.33102/mjsl.vol11no1.344.
[3] C. W. Pertiwi, B. Nuzulina, and B. Agricultural, "Exploring The Potential Of Halal Gelatin From Chicken By-Products : A Review," *J. Halal Prod. Res.*, vol. 8, pp. 13–21, 2025, doi: 10.20473/jhpr.vol.8-issue.1.13-21.
[4] D. E. Velazquez, "Effect of Organic Acid Mixtures on the Extraction Efficiency , Physicochemical , and Thermal Properties of Pigskin Gelatin and Resulting Films," *Physchem*, pp. 1–12, 2025, doi: https://doi.org/10.3390/physchem5030038.
[5] S. Cao, Y. Wang, and L. Xing, "Structure and Physical Properties of Gelatin From Bovine Bone Collagen Influenced by Acid Pretreatment and Pepsin," *Food Bioprod. Process.*, vol. 121, pp. 213–223, 2020, doi: 10.1016/j.fbp.2020.03.001.
[6] E. H. Lee *et al.*, "Optimized Collagen Extraction Process to Obtain High Purity and Large Quantity of Collagen from Human Perirenal Adipose Tissue," vol. 2022, no. 1, 2022, doi: 10.1155/2022/3628543.
[7] D. C. Valle, L. C. Quinapallo, and C. G. Galarza, "GELATIN EXTRACTION THROUGH ACETIC ACID USING TANNERY WASTE AS RAW MATERIAL," vol. 5, pp. 737–747, 2023.
[8] A. D. Hazmi, A. Faiza, M. Taib, S. Yahya, and N. Z. Alias, "Oxalic Acid from Averrhoa bilimbi L . as a Bleaching Agent," *Malaysian J. Chem.*, vol. 26, no. 5, pp. 407–416, 2024, doi: https://doi.org/10.55373/mjchem.v26i5.407.
[9] A. B. Sitanggang, "The Utilization of Bilimbi ( Averrhoa bilimbi ) and Lime ( Citrus aurantifolia ) Juices as Natural Acid Coagulants for Tofu Production," *J. Food Sci. Technol.*, vol. 57, no. 12, pp. 4660–4670, 2020, doi: 10.1007/s13197-020-04503-5.
[10] A. Azrita, E. Elfrida, and N. Aryani, "Functional Characterization of Gelatin From Freshwater Fish Scales Using Averrhoa bilimbi Acid Pretreatment," *Bioflux*, vol. 18, no. 2, pp. 822–835, 2025, doi: https://bioflux.com.ro/docs/2025.822-835.pdf.
[11] A. T. Rustan L.I, Sikanna R, "Karakterisasi Gelatin Berbasis Usus Ayam Broiler (Gallus Domesticus) Menggunakan Belimbing Wuluh (Averrhoa Bilimbi L.) Dengan Variasi Konsentrasi dan Waktu Ekstraksi," *J. Sainstiskom*, vol. 3, no. 1, pp. 18–28, 2025.
[12] P. K. Bhargavi, R. Banerjee, N. B. Maheswarappa, A. K. Verma, P. M. Govindaiah, and J. Lalthanmawii, "Sustainable Gelatin Extraction from Poultry Skin-Head-Feet Blend : An Ultrasound-Assisted Approach," *Poult. Sci.*, vol. 104, no. 4, p. 104975, 2025, doi: 10.1016/j.psj.2025.104975.
[13] Ç. Işık *et al.*, "Gelatin Extraction From Chicken Skin by Conventional and Ohmic Heating Methods and Comparison with Commercial Halal Gelatins," *Food Hydrocoll.*, vol. 150, no. December 2023, 2024, doi: 10.1016/j.foodhyd.2023.109694.
[14] N. . fernianti D, Juniar H,Adinda, "Pengaruh Massa Ossein dan Waktu Ekstraksi Gelatin dari Tulang Ikan Tenggiri dengan Perendaman Asam Sitrat Belimbing Wuluh," *Jourbal Distilasi*, vol. 5, no. 2, 2020, doi: https://doi.org/10.32502/jd.v5i2.3027.
[15] P. M. . Simpen N.I, "Karakteristik Mutu Gelatin dari Kulit Aya Broiler Melalui Proses Perendaman Kombinasi Asam-Basa," *J. Kim.*, pp. 204–211, 2016, doi: 10.24843/JCHEM.2016.v10.i02.p06.
[16] N. D. Mokhtar *et al.*, "Extraction Optimization and Characterization of Collagen from Chicken ( Gallus gallus domesticus ) Feet," pp. 79–85, 2017.
[17] C. Tan, A. A. Karim, U. Uthumporn, and F. C. Ghazali, "Effect of Extraction Temperature on the Physicochemical Properties of Gelatine from the Skin of Black Tilapia ( Oreochromis mossambicus )," vol. 30, pp. 1–21, 2019.
[18] P. M. Robert Gál, "Biotechnological Processing of Laying Hen Paw Collagen Into Gelatins," *Processes*, no. 1069, pp. 1–15, 2020.
[19] W. and L. G. W. L. Horwitz, "Official Methods of Analysis of AOAC INTERNATIONAL 18th Edition, 2005," *Off. Methods Anal. AOAC Int. 18th Ed. 2005*, no. d, pp. 4–5, 2005.
[20] C. E. Ozyurt, H. O. Yesilcimen, S. Mavruk, V. B. Kiyaga, and M. Perker, "Assessment of Some of the Feeding Aspects and Reproduction of S. undosquamis Distributed in the İskenderun Bay," *Turkish J. Fish. Aquat. Sci.*, vol. 17, no. 1, pp. 51–60, 2017, doi: 10.4194/1303-2712-v17.
[21] Z. Yang, X. Sha, H. Wang, T. Fang, S. Shu, and Z. Tu, "Effect of acid pretreatments with various acid types on gelling properties and identification characteristics of pigskin gelatin," *Food Chem. X*, vol. 26, no. January, p. 102211, 2025, doi: 10.1016/j.fochx.2025.102211.
[22] A. Kumar, C. Ali, M. P. Z. Sakhare, and N. Bhaskar, "Poultry Processing Waste as an Alternative Source for Mammalian Gelatin : Extraction and Characterization of Gelatin from Chicken Feet Using Food Grade Acids," *Waste and Biomass Valorization*, 2016, doi: 10.1007/s12649-016-9756-1.
[23] H. Jafari, A. Lista, M. M. Siekapen, and P. Gha, "Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering," *Polymers (Basel).*, pp. 1–36, 2020.
[24] N. D. Mahesti, H. M. Ansory, and A. Info, "Pemanfaatan Limbah Belimbing Wuluh , Kulit Jeruk , dan Bayam sebagai Sumber Asam Organik : Studi Konsentrasi Asam dan Potensi sebagai Agen Reduktor," *J. Farm. Indones.*, vol. 21, no. 2, pp. 167–174, 2024, doi: 10.31001/jfi.v21i2.2630.
[25] K. S. Sasmitaloka and A. Budiyanto, "Pengaruh Lama Waktu Perendaman Terhadap Karakteristik Gelatin Ceker Ayam," *Agrointek*, vol. 16, no. 2, pp. 179–185, 2022, doi: 10.21107/agrointek.v16i2.11846.
[26] K. Sockalingam, H. Z. Abdullah, and M. Izwana, "Effects of Acid Pre-Treatment on Removal of Organic and Inorganic Substances from Black Tilapia Bones and Scales," *Int. J. Integr. Eng.*, vol. 16, no. 5, pp. 26–33, 2024.
[27] S. Chand, S. K. Mendiratta, R. R. Kumar, S. Talukder, F. R. C. K, and P. Madane, "Gelatine Extraction from Chicken Skin as Influenced by NaOH Pretreatment," *J. Meat Sci*, vol. 16, pp. 37–42, 2021, doi: 10.5958/2581-6616.2021.00008.6.
[28] R. Sikanna, "Ekstraksi Gelatin dari Tulang Ikan Tongkol ( Euthynnus affinis ) dengan Menggunakan Asam Belimbing Wuluh ( Averrhoa bilimbi L )

[ Gelatin Extraction from Tongkol Fish Bone ( Euthynnus affinis ) using Acid," vol. 9, no. 1, pp. 85–91, 2023.

[29] R. J. Tangka, F. Mentang, and A. T. Agustin, "Pengaruh Perbedaan Konsentrasi Asam Asetat dan Lama Waktu Ekstraksi Kolagen dari Kulit Ikan Situhuk Hitam ( Makaira indica )," *Media Teknol. Has. Perikan.*, no. Sinta 4, pp. 44–49, 2020.

[30] R. Yenrina and M. Murti, "Antioxidant activity and characteristics of butterfly pea ( Clitoria ternatea ) syrup with the addition of Belimbing wuluh ( Averrhoa bilimbi . L ) juice," *World J. Adv. Eng. Technol. Sci.*, vol. 09, no. 02, pp. 338–344, 2023.

[31] K. J. G. S. J. Mccreath, "Investigation of the influence of pH on the properties and morphology of gelatin hydrogels," *J. Polym. Sci.*, no. March, pp. 2316–2332, 2023, doi: 10.1002/pol.20230141.

[32] J. Wang, "Chasing weakly-bound biological water in aqueous environment near the peptide backbone by ultrafast 2D infrared spectroscopy," *Commun. Chem.*, pp. 1–9, 2024, doi: 10.1038/s42004-024-01170-x.

[33] A. Prokopov, P. Mokrejš, G. Robert, and J. Pavlaˇ, "Characterization of Poultry Gelatins Prepared by a Biotechnological Method for Targeted Changes at the Molecular Level," *Int. J. Mol. Sci.*, pp. 1–17, 2024, doi: https://doi.org/10.3390/ijms25020916.