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Effect of phosphoric acid pretreatment on characterization of gelatin from broiler chicken (*Gallus gallus domesticus* L.) bones

D Yuliani*, A Maunatin, A Jannah and H H Fauziyyah

Chemistry Department, Science and Technology Faculty, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jalan Gajayana No. 50 Malang, 65144, Indonesia

*Corresponding author: dewi.yuliani@kim.uin-malang.ac.id

Abstract. Broiler chicken [*Gallus gallus domesticus* (Linnaeus, 1758)] bone is one of the alternative sources of collagen to form gelatin. This research was attempted to extract and characterize broiler chicken bone gelatin. Chicken bones were pretreated with phosphoric acid in various concentrations and curing times. The concentration of phosphoric acid was 8 %, 9 % and 10 %, while the curing time was 12 h and 24 h. Then, gelatin was extracted and physicochemical properties examined. Gelatin was pretreated by phosphoric acid 9 % for 24 h produced the best physicochemical properties with (15.4 ± 0.94) % of yield. Moisture, ash content, and pH of chicken bones gelatin were (3.96 ± 1.46) %, (24.0 ± 6.36) %, and (5.74 ± 0.003) %, respectively. Other physiochemical properties were (372.29 ± 4.62) g bloom of gel strength, (54.21 ± 1.24) % of protein content, and (60.21 ± 0.72) % of emulsion stability. Infrared spectra of chicken bones gelatin had the most with vibration peak at the wave number of $1\,639\text{ cm}^{-1}$ to the amide I, of $1\,545\text{ cm}^{-1}$ to the amide II, of $1\,129\text{ cm}^{-1}$ to the amide III, of $2\,292\text{ cm}^{-1}$ to the amide B, and $3\,456\text{ cm}^{-1}$ to the amide A.

Keyword: Alternative gelatin source, chicken bone waste, gelatin spectra, halal gelatin, physicochemical properties.

1. Introduction

Gelatin demand for industry in Indonesia has been increased annually. According to Ministry of Industry data in 2012, import of gelatin increased 20.26 % from 2007 to 2011. Central Bureau of Statistics Indonesia (2016) reported that enhancement of gelatin demand was from 3.149×10^3 t (2012) to 5.079×10^3 t (2016) [1]. Nowadays, gelatin is a popular ingredient obtained by partial hydrolysis or denaturation of collagen in animal raw materials [2, 3]. This compound has been applied in food and non-food industry such as cosmetic and pharmaceutical industry. Gelatin can be used as stabilizing, thickening and gelling agent [2, 4].

Indonesia import gelatin from USA, France, Germany, Brazil, South Korea, China and Japan. Source of commercial gelatin is mostly from porcine skin (46 %), bovine hides (29.4 %), bones (23.1 %) and other sources such as fish skin [5, 6]. Gelatin produced from porcine forbids for both Muslims and Jews because of religion reasons, while Hindu does not consume bovine-related products [7, 8]. The outbreak of mad cow disease or bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) causes attention as well because the diseases can be transmitted to human [9, 10]. Therefore, searching for alternative sources is importantly needed.

Broiler chicken [*Gallus gallus domesticus* (Linnaeus, 1758)] bones can be one of alternative gelatin sources. Broiler chicken is the largest poultry population in Indonesia and chicken meat consumption



produced bone waste as by-product [11]. The number of broiler chicken increased annually according to Directorate General of Animal Husbandry and Animal Health in 2017 [12]. Increasing of broiler chicken number leads to elevate bones waste.

The bone as by-product has become promising source of collagen and gelatin. Other potential and low cost of collagen sources such as poultry feet (chicken) and bones have risen to replace the mammalian and marine sources. Several previous researches have been studied gelatin extraction, but the research only focused on chicken feet [11, 13].

Utilization of chicken bone as gelatin source has not been studied intensively [14]. Previous studied researched about broiler chicken bones treated by various phosphoric acid concentrations (3 %, 5 %, and 7 %) for 24 h. Gelatin yield and gel strength of the research were in range 3.61 % to 5.89 % and 74.58 g to 145.06 g bloom, respectively [15]. The yield and the gel strength in that research were low and inappropriate with standard. Both parameters are crucial criteria for determination of commercial gelatin. In this study, broiler chicken bones were pretreated with 8 %, 9 %, 10 % of phosphoric acid to optimize the physicochemical properties of gelatin. The objective of this study was to extract and characterize broiler chicken bone gelatin.

2. Materials and methods

2.1. Materials

Broiler chicken bone was obtained from local market in Merjosari, Malang, East Java, Indonesia. The bones were mixture of sternum and femur of chicken. Chemicals used were phosphoric acid, commercial gelatin (bovine), and olive oil. All chemical reagents used were analytical grade.

2.2. Pretreatment on the broiler chicken bones

Broiler chicken bones were washed and ground into small pieces. The grounded bones were degreased with boiling for 30 min at 70 °C to remove the fat. Then, boiled bones were cleaned with tap water and dried at room temperature. Dried bones of 250 g were pre-treated by soaking in phosphoric acid (8 %, 9 % and 10 % (w/v)) at ratio of 1:4 (w/v) for 12 h and 24 h. Subsequently, the bones were washed with running tap water until they reached neutral pH. Treated bones were dried at room temperature [16].

2.3. Gelatin extraction

The extract procedure was conducted according to Widyasari and Rawdkuen [17] and Kaewdang et al. [18] with modification. Extraction gelatin from the treated bones was conducted using hot distilled water with ratio of 1:4 (w/v) at 55 °C to 75 °C in a water bath. Treated bones were extracted at 55 °C for 4 h. The mixture was filtered through cheesecloth to obtain the filtrate and remained bones as the residues. The procedure was repeated to the remained bones at 65 °C and 75 °C for 4 h. All of the filtrates at different temperature were collected in a container. The filtrate was sieved again using filter paper and the residue was evaporated at 50 °C for 2 h. The concentrated filtrate was poured into thin layers on plastic trays and oven dried at 50 °C for 24 h. Gelatin sheet were grounded and stored at 4 °C.

2.4. Determination of physicochemical properties of broiler chicken bone gelatin

2.4.1. Yield, moisture and ash content. Yield, moisture and ash content of chicken bone gelatin were determined using AOAC method [19].

2.4.2. Protein yield. Determination of protein yield was carried out by Kjeldahl method [20].

2.4.3. pH of gelatin. Gelatin of 0.2 g was dissolved at 20 mL of distilled water at 80 °C. The pH of the gelatin solution was measured by pH meter (Mettler Tolloedo) [21].

2.4.4. Gelatin gel strength. Distilled water was used to dilute gelatin to a solution concentration of 6.67 % (w/v). The solution was homogenized, and heat up to 80 °C for 15 min. The solution was put

into standard bloom jar (58 mm to 66 mm of diameter and 85 mm of height) and incubated at 10 °C for \pm 2 h. The gel strength was determined using a TA.XTPlus Texture Analyzer (Texture Analyzer Corp., Scarsdale, NY, USA). The sample was placed under a probe with 0.1923 cm² width and was pressed on a 97 g load. Calculation of gel strength used equation (1) and (2) [21].

$$D \text{ (dyne cm}^{-2}\text{)} = F/G \times 980 \quad (1)$$

$$\text{Gel strength (g bloom)} = 20 + [(2.86 \times 10^{-3}) \times D] \quad (2)$$

Where, F is height of curve, G is constanta, and D is gel strength (dyne cm⁻²).

2.4.5. Emulsion stability. Determination of emulsion stability was adopted by Hajrawati [22] procedure. Gelatin of 0.5 g was suspended with 12.5 mL of distilled water. The suspension was added 7.5 mL of olive oil, afterwards was blended for 2 min. The mixture was poured into beaker glass and was heat it up at 80 °C for 30 min. Emulsion stability is expressed as a mixture forming an emulsion after heating. This value was calculated using equation (3).

$$\text{Percentage of emulsion stability (w/w)} = \frac{\text{Mass of emulsion}}{\text{Mass of total mixture}} \times 100 \% \quad (3)$$

2.5. Fourier transform infrared (FTIR) analysis

The FTIR analysis was conducted according to Muyonga et al. [23]. Total 20 mg of gelatin was mixed with potassium bromide (100 mg) for the best physicochemical properties of gelatin and the spectrum was obtained using Shimadzu infrared spectrometer (Shimadzu FTIR-8400S) at 400 cm⁻¹ to 4 000 cm⁻¹.

3. Result and discussion

3.1. Gelatin extraction

Broiler chicken bones were pretreated with phosphoric acid before gelatin extraction. In the pretreatment, the bones were demineralized using 8 %, 9 %, and 10 % of phosphoric acid concentration with 12 h and 24 h of soaking time. Demineralization is a process to remove inorganic chemical components from the matrix bone. Al-Kahtani et al. [6] stated that bone demineralization depends on concentration of acid and soaking time.

Demineralization process produced swelling of the bones and released the organic collagens (ossein). The bone swelling is an important step for gelatin extraction due to protein in the bones was disrupted its non-covalent bonding and the collagen tends to solubilize [24, 25]. Figure 1 showed that swelling process increased weight of bone after demineralization. The higher concentration of phosphoric acid and the longer soaking time were obtained the higher ossein weight. At 12 h to 24 h of soaking time, weight of ossein was (369.35 \pm 4.90) g to (385.54 \pm 3.65) g, (397.20 \pm 1.66) g to (411.54 \pm 1.77) g, (399.91 \pm 2.51) g to (442.13 \pm 0.95) g for 8 %, 9 %, 10 % of phosphoric acid, respectively.

Enhancing of ossein weight indicated high intensity of interaction between phosphoric acid and matrix bone. The acid solubilizes inorganic compound in the bone and produced free collagen as an ossein. In the ossein form, collagen crosslink was partially cleaved to opened triple helix structure by weakening intermolecular, intramolecular and hydrogen bonds [26, 27]. The ossein was extracted using distilled water at 55 °C to 75 °C. In this extraction, opened triple helix structure transformed to single helix structure. Figure 2 presented the longer time of soaking gave the higher gelatin yield. For 12 h, phosphoric acid 8 %, 9 %, and 10 % pretreatment produced (8.53 \pm 1.39) %, (9.51 \pm 0.94) %, and (8.62 \pm 1.42) % of gelatin yield, respectively. For 24 h, percentage of gelatin yield was (13.4 \pm 1.26) %, (15.4 \pm 0.94) %, and (14.6 \pm 0.65) % for 8 %, 9 %, and 10 % of phosphoric acid concentration, in each.

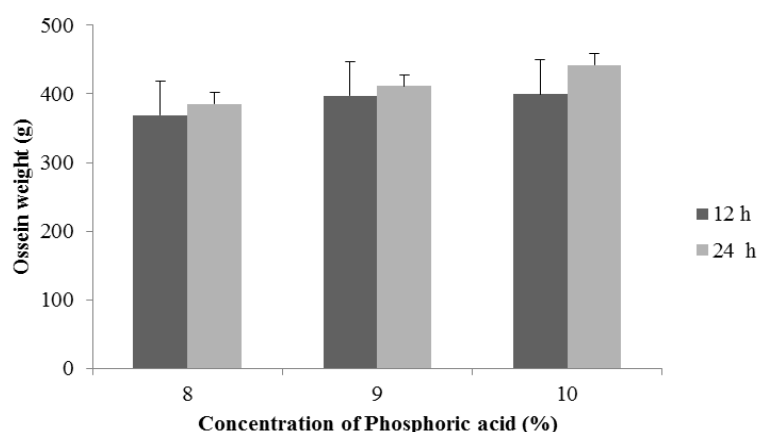


Figure 1. Weight of ossein from demineralization process by varied concentration of phosphoric acid (8 %, 9 %, 10 %) and soaking time (12 h, 24 h)

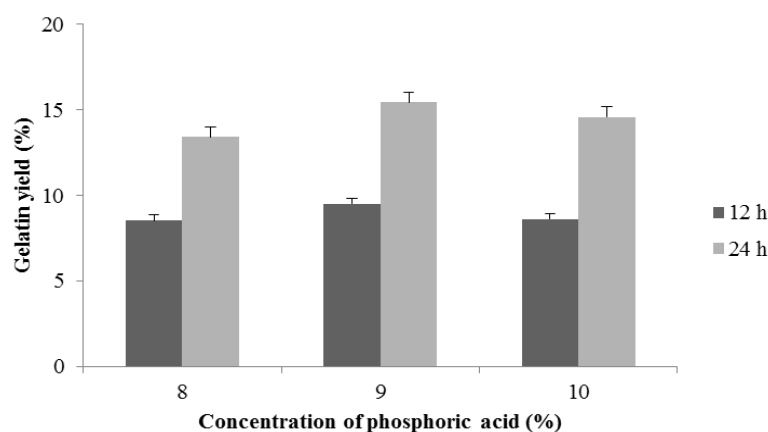


Figure 2. Gelatin yield of broiler chicken bones by phosphoric acid pretreatment in varying concentrations (8 %, 9 %, 10 %) and soaking time (12 h, 24 h)

Previous researches extracted gelatin from various raw material sources using phosphoric acid pretreatment resulted different yield. Extraction gelatin from chicken feet bone and cartilage produced 22.6 % of yield [28]. Widyasari and Rawdkuen [17] studied gelatin of chicken feet and the yield was 4.05 %.

3.2. Protein content

Protein is the main constituent of gelatin. Protein content of broiler chicken bone gelatin was showed in figure 3. From 12 h to 24 h of soaking time, protein content tends to decrease, especially for 8 % and 9 % of acid concentration. Reducing of protein content was $(48.63 \pm 4.09) \%$ to $(46.73 \pm 2.79) \%$ for 8 % acid pretreatment and $(58.79 \pm 4.06) \%$ to $(54.21 \pm 1.24) \%$ for 9 % acid pretreatment. Losing of gelatin may occur reducing protein content in the extraction process. Protein content at 10 % acid pretreatment was $(48.88 \pm 4.37) \%$ for 12 h to $(49.34 \pm 5.13) \%$ for 24 h.

In this study, broiler chicken bone gelatin produced protein content lower than other sources. Mulyani et al. [3] stated buffalo hide acid pretreatment produced $(85.96 \pm 0.51) \%$ to $(93.73 \pm 0.16) \%$ of protein content. Sompie et al. [29] researched protein content chicken skin feet by acid solution and resulted $(87.05 \pm 0.92) \%$ to $(88.93 \pm 0.79) \%$ of protein content. Amur sturgeon skin gelatin gave protein content 90.4 % [4].

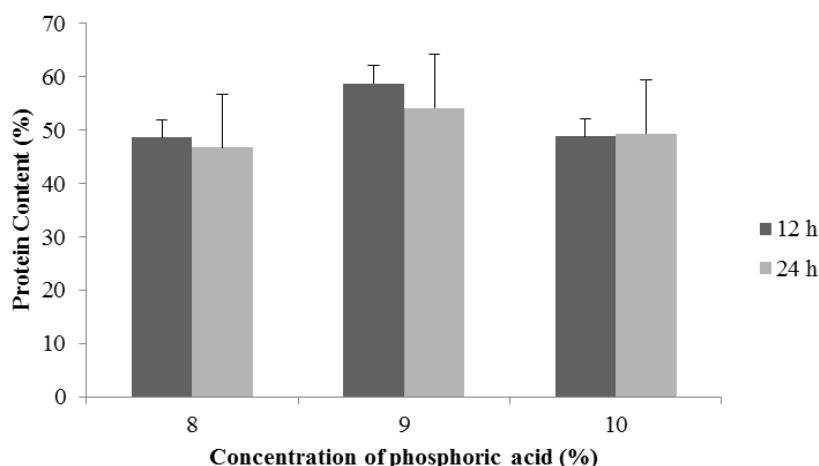


Figure 3. The protein content of broiler chicken bone gelatin.

3.3. pH value

The extracted broiler chicken bone gelatin showed acidic pH value due to the process involved. The pH values ranged from 5.71 ± 0.01 to 5.81 ± 0.01 was presented in figure 4. This value was higher than commercial gelatin (pH 4.5). According to British Standard Institute, range of pH was 4.5 to 6.5, while based on European Pharmacopeia, pH of gelatin standard is 3.8 to 7.6. Gelatin from tilapia (*Oreochromis niloticus* L.) bones produced pH of 4.23 ± 0.01 [28]. Rahman and Jamalulail [28] reported that chicken feet gelatin was extracted by acid pretreatment resulted pH of 6.15 ± 0.07 . The pH value of the gelatin solution obtained was influenced by washing treatment which is an important step to remove acid residues [30, 31].

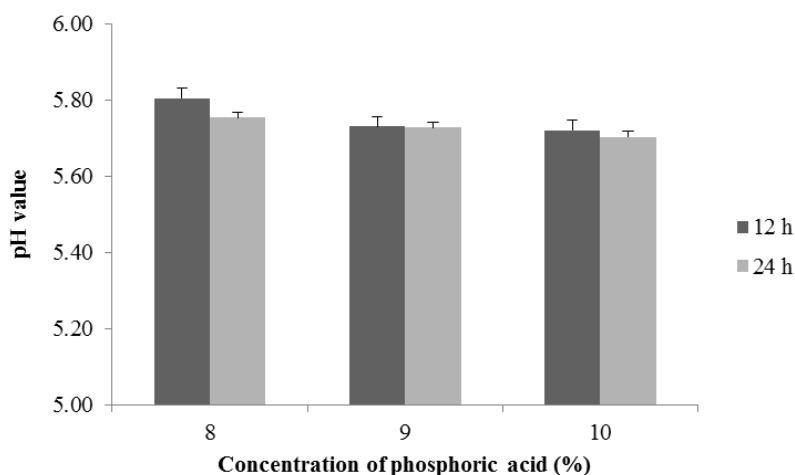


Figure 4. The pH value of broiler chicken bone gelatin.

3.4. Proximate analysis

The proximate composition of broiler chicken bone gelatin was tabulated in table 1. The moisture content indicated the presence of water in the sample. Range of moisture content of the gelatin for 12 h and 24 h was $(3.20 \pm 0.85) \%$ to $(5.18 \pm 0.27) \%$ and $(2.51 \pm 0.92) \%$ to $(3.96 \pm 1.46) \%$, respectively. This range fulfilled SNI No. 06-3736 and Gelatine Manufacture of Europe (GME) standard of moisture content that is 16 % and 15 %, respectively [20, 32]. Low moisture content was able to extent of drying and storage [33] and influenced the rheological properties such as elasticity and viscosity of the products [28].

Table 1. Effect of phosphoric acid treatment on moisture and ash content.

Phosphoric acid conc. (%)	Moisture content (%)		Ash content (%)	
	12 h	24 h	12 h	24 h
8	4.30 ± 0.43	2.51 ± 0.92	29.6 ± 3.59	30.7 ± 0.39
9	5.18 ± 0.27	3.96 ± 1.46	23.1 ± 1.57	24.0 ± 6.36
10	3.20 ± 0.85	2.63 ± 0.47	31.5 ± 0.56	27.3 ± 0.23

Ash content is one of parameters to determine quality of gelatin. In this study, the ash content of broiler chicken bone gelatin was higher than the gelatin standard. Broiler chicken bone gelatin has an ash content in the range (23.1 ± 1.57) % to (31.5 ± 0.56) % for 12 h and (24.0 ± 6.36) % to (30.7 ± 0.39) % for 24 h (table 1). Maximum ash content in gelatin based on SNI and GME was 3.25 % and 2 %, respectively. High ash content contributes to a low quality of broiler chicken bone gelatin. It was due to the gelatin may indicate that pretreatment, demineralization and extraction steps were effective in removing soluble minerals or the gelatin contains greater amount of calcium and other minerals in the bones [34, 35]. For future studies, measuring the bone ash content before extraction of gelatin is recommended.

Broiler chicken bone gelatin was compared to commercial gelatin and other gelatin sources. Moisture and ash content of commercial gelatin were found to be 12.48 % and 0.66 %, respectively. Kuan et al. [36] declared that acidic duck feet gelatin had (3.77 ± 0.47) % moisture and (2.47 ± 0.02) % ash content. Gelatin from chicken feet contained 6.64 % water and 1.56 % ash content [28].

3.5. Gel strength

Gel strength is the most important commercial criteria of a gelatin which determined based on hardness, stiffness, firmness, elasticity, strength, and compressive strength at the certain condition [37–39]. Table 2 presented the gel strength of the extracted broiler chicken bone gelatin. The gel strength range of broiler chicken bone gelatin was (409.72 ± 154.8) g to (428.41 ± 93.82) g bloom for 12 h and (368.35 ± 66.16) to (439.09 ± 201.4) g bloom for 24 h.

Table 2. Effect of phosphoric acid treatment on gel strength of gelatin.

Phosphoric acid conc. (%)	Gel strength (g bloom)	
	12 h	24 h
8	409.72 ± 154.8	368.35 ± 66.16
9	427.08 ± 77.67	372.29 ± 4.624
10	428.41 ± 93.82	439.09 ± 201.4

According to Gelatin Manufacturer Institute of America (GMIA) [40], high quality gelatin is 250 g to 300 g bloom gel strength, while commercial gelatin is 323.40 g bloom of gel strength. Gelatin from broiler chicken bones has gel strength higher than GMIA standard and gelatin commercial. Gelatin with gel strength higher than 300 g bloom gave rigid and hard texture. Gel strength of gelatin from broiler chicken bones has similarity property to Hermanto et al research. The research reported that cattle fish gelatin resulted 416.57 g bloom gel strength [16]. Broiler chicken bone gelatin gave different result with Kuan et al. [36] and Rahman and Jamalulail [28]. Duck feet gelatin and bovine gelatin produced (209.63 ± 5.29) g and (232.63 ± 2.01) g bloom, respectively [36], and chicken feet gelatin produced 264.3 g bloom of gel strength [28].

3.6. Emulsion stability

Emulsifying properties is used to investigate gelatin performance to form emulsion system. Standar Nasional Indonesia (SNI) and GME do not require standard of emulsion stability. Commercial gelatin has 47.35 % emulsion stability. In this study, broiler chicken bone gelatin yielded emulsion stability higher than commercial gelatin (figure 5). The figure showed broiler chicken bone gelatin at 24 h was higher than that of at 12 h. The higher of phosphoric acid concentration led to higher of emulsion

stability. From 12 h to 24 h, emulsion stability of gelatin was $(9.820 \pm 1.67) \%$ to $(57.46 \pm 1.82) \%$, $(13.11 \pm 2.23) \%$ to $(60.21 \pm 0.72) \%$, $(15.46 \pm 2.16) \%$ to $(61.19 \pm 2.32) \%$ for 8 %, 9 %, 10 % of phosphoric acid, respectively.

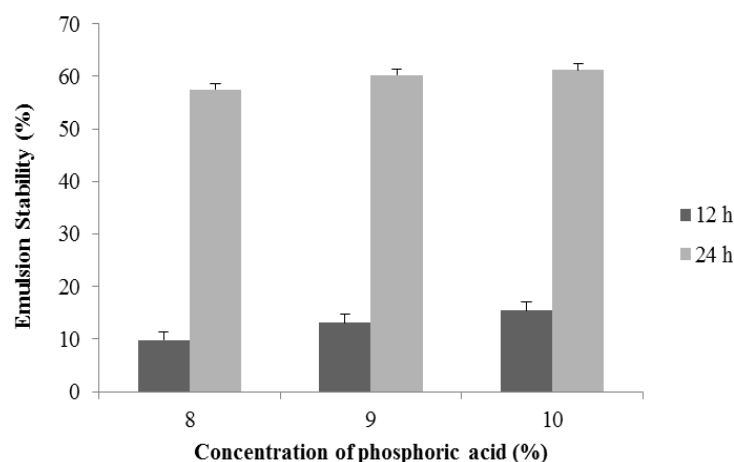


Figure 5. The emulsion stability of broiler chicken bone gelatin.

3.7. FTIR analysis

FTIR spectrum has been used to monitor the functional groups and secondary structure of gelatin [23]. The FTIR spectra of broiler chicken bone gelatin by 9 % of phosphoric acid treatment was shown in figure 6. It can be seen in the spectra, gelatin sample contained major peaks in the amide region.

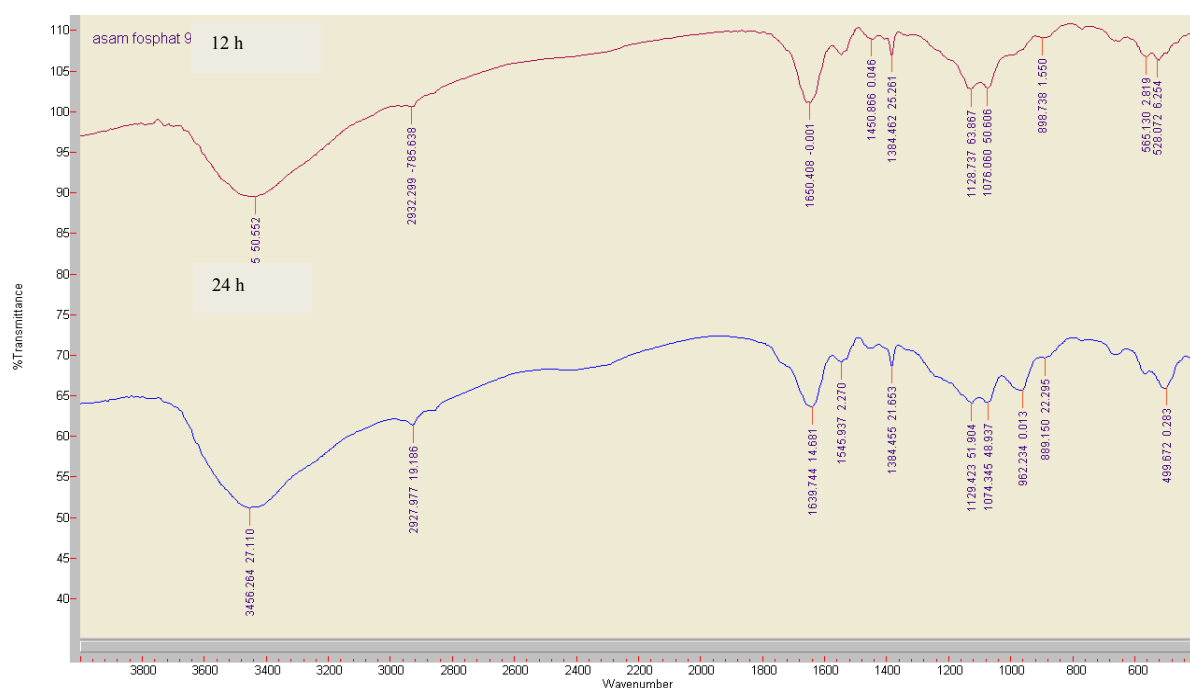


Figure 6. Infrared spectra of broiler chicken bone gelatin at 12 h and 24 h of phosphoric acid pretreatment.

Gelatin was pretreated for 12 h and 24 h showed five peaks were detected in the amide region. For 24 h of pretreatment, the peaks were amide I (1639 cm^{-1}), amide II (1545 cm^{-1} to 1384 cm^{-1}), amide III (1129 cm^{-1} to 1074 cm^{-1}), amide A (3456 cm^{-1}), and amide B (2927 cm^{-1}). For 12 h of

pretreatment, broiler chicken bone gelatin gave the vibration peaks at the wavenumber $1\,650\text{ cm}^{-1}$ to the amide I, $1\,450\text{ cm}^{-1}$ to $1\,384\text{ cm}^{-1}$ to the amide II, $1\,128\text{ cm}^{-1}$ to $1\,078\text{ cm}^{-1}$ to amide III, $3\,437\text{ cm}^{-1}$ to the amide A, and $2\,932\text{ cm}^{-1}$ to the amide B. FTIR spectra of both gelatin was not significantly different. The peaks are similar to the chicken feet gelatin [17, 41], and thus, indicated the similarity of the corresponding functional group.

4. Conclusion

Gelatin from broiler chicken bones produced the best of physicochemical properties at phosphoric acid 9 % for 24 h. Gelatin yield was obtained $(15.4 \pm 0.94)\%$. Water content, ash content, and pH of the gelatin were $(3.96 \pm 1.46)\%$, $(24.0 \pm 6.36)\%$, and $(5.74 \pm 0.003)\%$, respectively. Other physicochemical properties were $(372.29 \pm 4.62)\%$ of gel strength, $(54.21 \pm 1.24)\%$ of protein content, and $(60.21 \pm 0.72)\%$ of emulsion stability. Based on Indonesia National Standard (SNI), water content and pH value fulfilled the requirement, but ash content was out of the requirement. Value of gel strength is higher than British Standard gave rigid and solid texture. Infrared spectra of chicken bones gelatin showed five vibration peak at the wavenumber of $1\,639\text{ cm}^{-1}$ to the amide I, of $1\,545\text{ cm}^{-1}$ to the amide II, of $1\,129\text{ cm}^{-1}$ to the amide III, of $2\,292\text{ cm}^{-1}$ to the amide B, and $3\,456\text{ cm}^{-1}$ to the amide A.

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