Sustainable valorization of poultry by-products: Optimized extraction of gelatin from chicken feet using acid treatments

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ABSTRACT

Chicken feet, a major by-product of poultry slaughterhouses, are underutilized despite their high collagen content, which can be converted into gelatin. This study aimed to optimize gelatin extraction from chicken feet using hydrochloric acid (HCl) and phosphoric acid through Response Surface Methodology (RSM), focusing on yield, pH, lightness (L*), and viscosity. Extraction conditions were varied by acid concentration and soaking duration. Results showed that prolonged extraction time significantly improved yield, while excessively high acid concentrations led to over-hydrolysis and reduced recovery. Optimized conditions for HCl were achieved at 2.09% concentration and 19.11 h soaking time with a yield of 8.45% (desirability 0.986), while phosphoric acid achieved optimal results at 11.06% and 21.51 h with a yield of 8.61% (desirability 1.00). Gelatin quality was strongly affected by pH, where near-neutral conditions enhanced structural stability. Lightness decreased with extended soaking, whereas viscosity was higher in phosphoric acid treatment compared to HCl. This research highlights the sustainable valorization of poultry by-products into value-added gelatin, supporting circular economy principles and offering an alternative source of functional biopolymer for food applications.

Introduction

Chicken feet is one of the by-products of poultry slaughterhouses (RPA), with limited utilization primarily due to their low meat content. According to the latest data from the Directorate General of Livestock and Animal Health (Humaspkh, 2024) Indonesia's chicken production reached 3.84 million tons, while the national demand for 2024 was projected at only 3.72 million tons. While Indonesia has achieved self-sufficiency in poultry commodities, particularly chicken, significant challenges persist in the utilization and processing of chicken feet as a by-product of poultry slaughtering. Chicken feet consist of several components, including bones, skin, muscles, connective tissues, and collagen, which accounts for 30-35% of their composition (Kuan et al., 2017). Furthermore, several specific amino acids had been identified, were 35% glycine, 11% alanine, and a significant amount of proline (Almeida and Lannes, 2013). Collagen is a source of gelatin and used in many food industries, either as an ingredient of food itself or as coating material. Building upon this potential, the process of gelatin extraction from chicken feet can utilize various acid sources, including acetic acid, hydrochloric acid, citric acid, and lactic acid. Hydrochloric acid (HCl) and phosphoric acid (H₃PO₄) are used in collagen breakdown for gelatin extraction, with varying effectiveness influenced by their chemical properties and application conditions. Previous researches have been discussed the use of gelatin derived from chicken feet's collagen. Most of studies applied only one acid to explore the effectiveness of each acid. Meanwhile, several acids have their own potentials to be used to extract gelatin. A study conducted by Fatima et al. (2022) which studied about the effect of various variables (acetic acid concentration, extraction temperature, and extraction time) and only used one acid to extract gelatin and used it as edible coating, while another research also explored only one acid with various concentrations (Arina et al., 2021; Rohman et al., 2024). There are still limited research that discussed every acid with their potential. By exploring the potential in every acid solution using

Response Surface Methodology (RSM), this study identified the optimal conditions for each acid.

Materials and methods

This study was conducted from April to August 2025 at the Laboratory of Food Chemistry and Nutrition and the Laboratory of Food and Agricultural Product Engineering, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia. Fresh chicken feet (broiler, female, approximately 6 weeks old, 1.5–2.0 kg, obtained from a traditional market in Semarang, Indonesia) were used as raw materials. Chemicals included distilled water (Brataco, Jakarta, Indonesia), hydrochloric acid (HCl, p.a., Merck KGaA, Darmstadt, Germany), and phosphoric acid (p.a., Merck KGaA, Darmstadt, Germany). Equipment included an analytical balance (Shimadzu AUW220D, Kyoto, Japan), dehydrator (Ezidri FD770, Christchurch, New Zealand), filter cloth, stainless steel knife, volumetric flask, measuring cylinder, beaker glass, magnetic stirrer (Scilogex MS300HS, Rocky Hill, CT, USA), grinder (Philips HR2116, Jakarta, Indonesia), pH meter (Hanna HI2211, Woonsocket, RI, USA), water bath (Memmert WNB7, Schwabach, Germany), refrigerator (Panasonic NR-BL307, Osaka, Japan), stainless steel spoon (Kris Chef, Jakarta, Indonesia), dropper pipette, color reader (Konica Minolta CR-400, Tokyo, Japan), and viscometer (Brookfield DV-E, Middleboro, MA, USA).

The research began with preliminary extraction of gelatin from chicken feet, cleaned and chopped samples (~5 cm) were ground and soaked in acid solutions (HCl and phosphoric acid) with varying concentrations and soaking durations. After acid treatment, samples were filtered, washed repeatedly with distilled water until reaching neutral pH, soaked in water at a 1:2 (b/v) ratio, and extracted in a water bath (60°C, 5 h). The gelatin solution was filtered and dried using a dehydrator to obtain gelatin powder. Gelatin was then analyzed for yield (percentage of dried gelatin relative to initial raw material), lightness (L*) using a color read-

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er (Konica Minolta CR-400), pH measured by dissolving gelatin at 6.67% (b/v) in distilled water, heating at 60°C for 15 min, cooling to 25–30°C, and measuring with a pH meter (Hanna HI2211), and viscosity measured directly afterward with a Brookfield viscometer at 30 rpm. Experimental design and data analysis were carried out using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Acid concentration and soaking time were set as independent variables, while yield, lightness (L*), pH, and viscosity were considered as response variables. Statistical analyses and model fitting were performed using Minitab 18 software (Minitab, LLC, State College, PA, USA). The parameters of the RSM analysis are presented in Tables 1 and 2.

Table 1. Treatment of HCl acid.

Std Order	Run Order	Pt Type	Blocks	Concentration (%)	Duration (hour)
8	1	-1	1	1.75	26.49
12	2	0	1	1.75	18
4	3	1	1	3	24
1	4	1	1	0.5	12
7	5	-1	1	1.75	9.51
6	6	-1	1	3.52	18
10	7	0	1	1.75	18
2	8	1	1	3	12
3	9	1	1	0.5	24
11	10	0	1	1.75	18
9	11	0	1	1.75	18
13	12	0	1	1.75	18
5	13	-1	1	-0.02	18

Table 2. Treatment of phosphate acid.

	Std Order	Run Order	Pt Type	Blocks	Concentration (%)	Duration (hour)
P4	7	1	-1	1	7.5	21.51
P2	3	2	1	1	5	36
P5	8	3	-1	1	7.5	38.49
P1	9	4	0	1	7.5	30
P9	2	5	1	1	10	24
P7	5	6	-1	1	3.96	30
P1	10	7	0	1	7.5	30
P1	13	8	0	1	7.5	30
P6	1	9	1	1	5	24
P3	6	10	-1	1	11.04	30
P1	12	11	0	1	7.5	30
P1	11	12	0	1	7.5	30
P8	4	13	1	1	10	36

Results

Yield

HCI treatment

Response surface analysis (Fig. 1) showed that extraction duration had a greater influence on yield compared to HCl concentration. The quadratic model obtained (Y= $-16.12-1.22X_1+2.704X_2-0.416X_1^2-0.079X_2^2+0.1547X_1X_2$) indicated that increasing extraction time generally enhanced yield, although the increment tended to plateau at longer durations. In contrast, the effect of concentration was more variable. Linearly, higher concentrations tended to reduce yield; however, its interaction with duration revealed a positive effect, suggesting that the combination of moderate concentration and prolonged duration could produce higher yields.

The contour plot illustrated that the optimum yield region was achieved at longer durations (approximately 16–20 h) and moderate concentrations (around 2%). This pattern was further supported by the surface plot, which showed a steady increase in yield with prolonged duration until reaching the optimum point, followed by stabilization. Based on optimization results, the best condition was obtained at 2.089% concentration and 19.11 h extraction time, with a predicted maximum yield of 8.45% and a desirability value of 0.986, which is very close to 1. This indicates a strong agreement between the model and the experimental data.

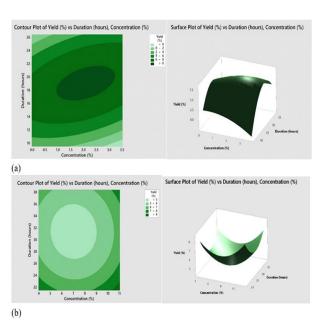


Fig. 1. Contour Plot and Surface Plot of Yield, (a) HCl; and (b) Phosphate.

Phosphate treatment

Response surface analysis (Fig. 1) showed that both concentration and extraction duration had a significant effect on phosphate yield. The obtained quadratic model (Y = $33.5 - 1.78X_1 - 1.447X_2 + 0.1208X_1^2 + 0.0227X_2^2 - 0.0025X_1X_2$) indicated that an increase in concentration and duration within a certain range could enhance the yield; however, when the values exceeded the optimum range, the yield decreased. This trend was evident in the contour plot, which displayed concentric circular patterns with an optimum area at medium concentration ($\approx 11\%$) and moderate duration (≈ 21 h), while the surface plot revealed a peak point of yield formed due to the interaction between the two variables. Based on the optimization results, the best condition was achieved at 11.06% concentration and 21.51 h duration, with a predicted maximum phosphate yield of 8.61% and a desirability value of 1.00, indicating an excellent agreement between the model and the experimental data.

ΔpH

HCI treatment

The response surface analysis (Fig. 2) revealed that HCl concentration had a more significant effect on the final pH compared to immersion duration. The quadratic regression model derived from the interaction between HCl concentration and immersion duration was (Y= 0,26 – 0,01X₁ – 0,095X₂ + 0.091X₁² + 0,00193X₂² – 0,02277X₁X₂). This equation indicates that excessively low or high HCl concentrations result in a greater ΔpH , whereas at moderate concentrations with certain durations, a smaller ΔpH is obtained. This condition is clearly reflected in the contour plot, where lighter areas represent smaller ΔpH values, while darker areas indicate higher ΔpH values. The optimization results showed that the best condition was achieved at a concentration of approximately 3.3749% and a duration of 26.4853 minutes with ΔpH = 0.4038, which means that the

gelatin pH approached 7, or neutral.

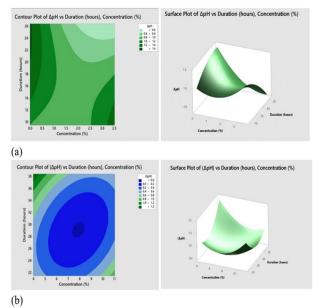


Fig. 2. Contour Plot and Surface Plot ΔpH (a) HCl: and (b) Phosphate.

Phosphate treatment

Based on the response surface analysis (Fig. 2), it was shown that the concentration of phosphoric acid had a greater effect on pH changes compared to the soaking duration. This can be observed in the obtained quadratic regression equation:

 $\Delta pH = 5.01 - 0.264X_1 - 0.273X_2 + 0.0334X_1^2 + 0.00595X_2^2 - 0.00950 X_1X_2$

The surface plot indicating that both excessively low and high concentrations lead to an increase in ΔpH . Meanwhile, the contour plot clearly shows that the darker areas correspond to higher ΔpH values, while the lighter areas indicate lower ΔpH values. The contour plot also reveals an optimum zone in an elliptical shape at the center, suggesting that a specific combination of phosphoric acid concentration and soaking duration can produce a minimum ΔpH , approaching neutrality. The most optimal condition was found at a phosphoric acid concentration of 7.8928% and a duration of approximately 29.23 hours, yielding a ΔpH of 0.0085, which indicates a nearly neutral pH with a desirability value of (d = 1.000).

L* (Lightness)

HCI treatment

The response surface analysis (Fig. 3) showed that the L* parameter (lightness) was influenced by HCI concentration and soaking duration with different levels of significance. The quadratic model obtained (L*= $87.4 + 0.03X_1 - 1.03X_2 + 0.85X_1^2 + 0.0277X_2^2 - 0.128X_1X_2$) indicated that increasing the concentration (X₁) generally tended to increase the L* value, although its effect was relatively smaller compared to the influence of duration (X2). Excessively long soaking times led to a decrease in lightness, which was likely due to the degradation of natural pigments and the formation of darker hydrolysis products. The contour plot revealed an optimum area at medium to high concentrations with moderate durations, where the L* values reached their maximum range. Meanwhile, the surface plot confirmed the response pattern, showing that the combination of high concentration with prolonged duration actually reduced lightness, making extraction time control a critical factor in maintaining color. Based on the optimization results, the best condition was achieved at an HCl concentration of 3.52% with a soaking duration of 9.51 hours, yielding a predicted maximum L* value of 86.42 with a desirability of 1.000. This desirability value indicates an excellent fit between the model and the experimental data, making the prediction highly reliable.

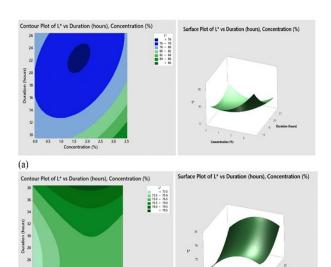


Fig. 3. Contour Plot and Surface Plot L* (a) HCl: and (b) Phosphate.

Phosphate treatment

The response surface analysis (Fig. 3) indicates that the L* (lightness) parameter is governed by both phosphate concentration (X₁) and soaking duration (X₂), with concentration exerting the stronger effect. The fitted quadratic model, (L= $79.3 + 3.16X_1 - 1.284X_2 - 0.1746X_1^2 + 0.0255X_2^2 -$ 0.0057X₁X₂,) shows a positive linear effect of phosphate concentration and a negative quadratic term, implying that L* increases with phosphate concentration up to a point and then declines at higher levels, consistent with excessive phosphate treatment that can cause structural changes and darker color formation. Duration exhibits a negative linear effect with a small positive quadratic term, suggesting lightness decreases as soaking time increases from short to intermediate durations, with a slight recovery at very long times. The negative interaction (-0.0057X₁X₂) indicates that simultaneously high concentration and long duration tend to reduce lightness more than either factor alone. Contour and surface plots derived from this model reveal an optimum region at high phosphate concentration with extended duration, after passing through the mid-duration trough.

Viscosity

HCI treatment

The viscosity values for the hydrochloric acid (HCl) treatment (Fig. 4) were in the range of 2.2–2.8 cP. HCl acts mainly as a hydrolyzing agent that breaks down the collagen structure, resulting in a less viscous solution.

Phosphate treatment

The viscosity values (Fig. 4) for the phosphoric acid treatment were consistent at around 3.2 cP. As a weak acid, phosphoric acid produced relatively higher viscosity values compared to hydrochloric acid, which is classified as a strong acid.

Discussion

The findings demonstrated that acid type, concentration, and soaking duration strongly influenced gelatin properties. For yield, extraction duration was the dominant factor in HCl treatment, aligning with Bahar et

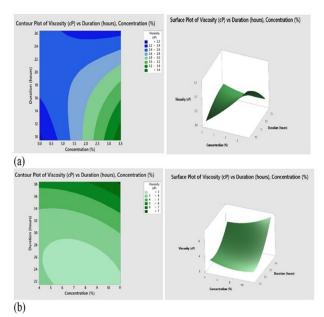


Fig. 4. Contour Plot and Surface Plot Viscosity (a) HCl: and (b) Phosphate.

al. (2020) and Nurilmala et al. (2023), who showed that longer extraction time enhances compound release. However, excessively high acid concentrations reduced yield, consistent with Mulyani et al. (2017), due to over-hydrolysis and loss of small protein fragments. Phosphoric acid treatment exhibited a clearer peak, where both variables needed to be balanced at medium levels for optimal yield. The soaking time and acid concentration are related to the yield produced. According to Bahar et al. (2020), a longer extraction time provides more opportunity to maximize the amount of gelatin compounds extracted. Similarly, Nurilmala et al. (2023) reported that yield significantly increased when the extraction time was extended from 6 to 8 h. On the other hand, excessively high concentrations resulted in a decrease in yield. This finding is consistent with Mulyani et al. (2017), who observed that yield increased at HCl concentrations of 0.3-0.9 M but declined at concentrations above 0.9 M. The reduction in yield may be attributed to over-hydrolysis, which generates excessively small protein fragments that are easily lost during washing. Therefore, the optimum condition occurs at a balance point, where the solvent concentration is sufficient to maximize compound release, and the extraction duration is not excessively long to minimize degradation.

For ΔpH , both acids showed strong dependence on concentration. HCl caused sharper changes because of its stronger ionization, while phosphate acid displayed a U-shaped curve where extreme concentrations increased ΔpH. Maintaining ΔpH near neutral is critical for gelatin stability, as supported by Nagarajan et al. (2015) and Islam et al. (2020), who emphasized that gelatin properties improve when pH approaches neutrality. This finding is consistent with the study conducted by Nagarajan et al. (2015), which demonstrated that the mechanical properties of gelatin reached their optimal quality when the pH was close to neutral, since under this condition the interactions among polypeptide chains of gelatin become more balanced, thereby stabilizing the matrix network. Consequently, this enhances the stiffness and tensile strength of the gelatin film. Hydrochloric acid (HCl) is a strong acid that ionizes completely in solution, so even small changes in its concentration can cause significant changes in H⁺ ions, which rapidly alter the pH. The higher the concentration of HCl, the lower the pH of the solution, or the greater the Δ pH value. Gelatin quality improves when the ΔpH value is smaller, meaning the pHis closer to neutral. Research conducted by Islam et al. (2020) showed that at neutral pH, the protein bands α -, β -, and γ - are better preserved, thereby minimizing protein degradation. In contrast, highly acidic or alkaline conditions lead to degradation due to excessive hydrolysis of peptide bonds in collagen. This indicates that treatment with an appropriate concentration of HCl is a crucial factor in obtaining good-quality gelatin, as it helps maintain the solution's pH closer to neutral, which is the optimal condition for preserving protein structure and quality.

The result of ΔpH in phosporic acid is consistent with the study by Araujo *et al.* (2015) on fish gelatin, which also achieved a desirability value of 1 under optimal conditions. This indicates that the optimized parameters were successfully achieved, resulting in gelatin of optimal quality.

The higher the concentration of phosphoric acid used during the soaking process, the greater its effect on lowering the pH. A study by Saenmuang *et al.* (2019) reported that the pH of gelatin could range from 3.7 to 4.8 due to insufficient washing, which allows acid residues to remain in the collagen matrix and dissolve during extraction, resulting in more acidic gelatin. Gelatin solutions exhibit structural weakness under acidic conditions. Goudie *et al.* (2023) revealed that gelatin becomes soft at very low pH (pH 2) due to an excess of H⁺ ions, which disrupt hydrogen bonds between protein chains that maintain gel strength. Conversely, gelatin solutions are stronger and more stable when the pH is close to their isoelectric point (pH 5–10).

For the lightness (L*), prolonged soaking generally reduced brightness due to pigment degradation and non-enzymatic browning, as reported by Bichukale (2018) and Nath et al. (2022). HCl treatment produced higher maximum L values, while phosphate treatment required relatively high concentrations and longer durations to achieve optimal brightness. These results confirm that color quality is highly dependent on controlling both concentration and time. Optimization yielded in phosphate treatment are, $X_1 \approx 8.3928\%$ and $X_2 \approx 38.4853$ h, giving a predicted maximum L= 80.0433* with desirability d = 1.000, which reflects an excellent agreement between the model and the experimental data within the explored factor space. Practically, these results highlight that controlled, relatively high phosphate concentration (~8-8.5%) combined with long soaking (~38-39 h) can maximize brightness, while avoiding the combination of very high concentration and prolonged time that would otherwise intensify non-enzymatic browning. Careful control of phosphate treatment conditions is therefore critical to obtaining a product with optimal, bright color. Several studies support this observation. Nuñez et al. (2023) noted that gelatin color is significantly influenced by both the raw material source and extraction conditions, which in turn affect consumer acceptance. Consumer perception of food quality attributes, including visual appearance and texture, is also a major determinant of product preference (Tamimi et al., 2025). Bichukale, (2018) reported that prolonged pretreatment increases non-enzymatic browning and reduces L* values, though color changes do not affect the functional properties of gelatin. Shyni et al. (2014) further highlighted that drying methods also influence gelatin color, with microwave-dried gelatin showing the highest brightness (L*). Similarly, Nath et al. (2022) explained that non-enzymatic browning results from reactions between protein amine groups and reducing sugars or carbonyl compounds, which intensify as these compounds accumulate.

For viscosity, HCI produced lower values due to stronger hydrolysis, in line with Gumilar *et al.* (2023) and Juliasti *et al.* (2015). The breakdown of collagen chains is influenced by the amount of hydrochloric acid used during the dehairing process. Insufficient HCI leads to incomplete collagen degradation, whereas higher concentrations promote more extensive structural changes, which in turn affect viscosity values (Gumilar *et al.*, 2023). Lower viscosity may also be attributed to collagen conditioning, where cleavage of peptide bonds occurs in the primary structure of weakly cross-linked chicken collagen (Prokopová *et al.*, 2022). Furthermore, increasing HCI concentration can cause the unfolding of amino acid chains, producing shorter chains and thereby decreasing viscosity (Juliasti *et al.*, 2015). Overall, HCI demonstrates a stronger hydrolyzing effect that disrupts collagen structure more extensively, leading to lower viscosity compared to phosphoric acid.

Phosphate treatment maintained higher viscosity, consistent with da Silva *et al.*, (2021) who noted that longer collagen chains correlate with higher viscosity. An increase in phosphoric acid concentration may enhance the formation of dissolved solids, contributing to a slight rise in solution viscosity, although the effect is not highly significant. Bichu-

kale (2018) reported the viscosity of chicken skin gelatin in the range of 3.83–5.53 cP at 60°C. This result is not substantially different from our findings, which showed a viscosity range of 2.6–4.0 cP for the phosphoric acid treatment. Gelatin with higher viscosity generally contains longer collagen chains, which increases flow resistance compared to gelatin with lower viscosity (Gál *et al.*, 2020). Similarly, higher molecular mass in gelatin is associated with higher viscosity values (da Silva *et al.*, 2021).

Overall, HCI treatment favored lighter gelatin with lower viscosity, while phosphate treatment favored more viscous gelatin with slightly lower lightness. The choice between treatments should therefore depend on the desired functional characteristics of the final gelatin product. Building upon these findings, future studies should focus on process optimization by integrating technological systems that enable real-time control of extraction variables and quality parameters. Such integration would enhance monitoring precision and production consistency under standardized food manufacturing systems (Tamimi and Pratama, 2025). Implementing this approach could lead to scalable and automated gelatin production frameworks that support sustainable utilization of poultry by-products while maintaining high quality assurance across the food processing sector.

Conclusion

This study demonstrated that chicken feet, an underutilized by-product of the poultry industry, can be sustainably valorized as a source of gelatin. The type of acid, concentration, and soaking duration significantly influenced the physicochemical properties of gelatin, including yield, pH, lightness, and viscosity. Hydrochloric acid treatment resulted in lighter-colored gelatin with lower viscosity, while phosphoric acid treatment produced gelatin with higher viscosity but slightly lower brightness. Optimal extraction conditions were identified for both acids using Response Surface Methodology, achieving high yields and desirable quality parameters. These findings highlight the potential of converting poultry by-products into value-added biopolymers, supporting circular economy practices and contributing to sustainable food ingredient development.

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Conflict of interest

The authors have no conflict of interest to declare.

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