Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Modeling and optimization of different chicken feet gelatin extraction processes using Response Surface Methodology

Hanieh Esmaeili [1], Reza Farahmandfar[2], Ali Motamedzadegan[2], Maryam Asnaashari[3*]

1-MSc, Department of Food Science and Technology, Sari Agricultural Sciences & Natural Resources University (SANRU), Sari, Iran

2-Professor, Department of Food Science and Technology, Sari Agricultural Sciences & Natural Resources University (SANRU), Sari, Iran

3-Assistant Professor, Department of Animal Processing, Animal Science Research Institute of Iran (ASRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

ARTICLE INFO

Article History:

Received:2025/03/18

Accepted:2025/08/16

Keywords:

Chicken feet,

Response surface,

Efficiency,

Gel strength,

Optimization

DOI: 10.48311/fsct.2026.84003.0

*Corresponding Author E-

m.asnaashari@yahoo.com

ABSTRACT

The consumption of animal by-products has grown significantly in recent decades. These by-products can be converted into sustainable products for agricultural and industrial uses. One of these sustainable products is gelatin. Among poultry slaughterhouse wastes, chicken feet are a good source for gelatin production, but there are limited studies on it. In this study, in order to determine the optimal conditions for gelatin extraction from chicken feet, the effects of four variables: sulfuric acid concentration (1 to 3 N), weight ratio of kneaded chicken feet to acid solution (1: 2-6 W/V), temperature (60 to 90°C), and extraction time (1 to 5 hours) by the acid method and three variables: sulfuric acid concentration (1 to 3 N), microwave power (360 to 720 W), and microwave time (10 to 20 min) by the microwave method on the yield and strength of the gel were investigated, respectively. The results of the experiments were analyzed using the response surface central composite design method. The proposed quadratic models for extraction efficiency and gel strength in the acid and microwave methods had high R^2 and Adj-R^2 and no significant lack of fit, so they were able to predict the responses well. The optimal conditions for acid extraction for maximum efficiency and gel strength were determined as temperature 76.40°C, 3 hours, 2 N acid and weight ratio of kneaded chicken feet to acid solution 1:4 W/V. Analysis of variance showed the microwave extraction process at 2 N acid concentration, power 540 W and time 20 min resulted in the highest efficiency and gel strength. Then, the optimal samples of both methods with commercial bovine gelatin were examined in terms of acrylamide gel electrophoresis and color. The results showed that chicken feet gelatin can be a suitable alternative to mammalian gelatin.

1-Introduction

Poultry processing industries around the world produce large amounts of by-products such as heads, feet, bones, giblets and feathers. These wastes are often processed into animal feed, fertilizer and pet food or simply discarded. Improper disposal of these wastes causes environmental pollution, the emergence of various diseases and the loss of useful biological resources such as proteins, enzymes and lipids [1]. The poultry industry produces large quantities of by-products such as chicken feet each year. These by-products contain relatively high levels of protein (especially collagen) and are used to produce gelatin. Gelatin is a biopolymer with unique properties that has a wide range of applications in the food, pharmaceutical, and photographic industries [2In the past, most commercial gelatins were derived from mammalian sources such as pigs and cows, but due to cultural and religious restrictions, frequent outbreaks of diseases (such as bovine spongiform encephalopathy)[1](BSE) led some studies to investigate the replacement of collagen from different animal sources such as aquatic and poultry [3, 4Although much research has been conducted on the production of gelatin from fish, fish gelatin accounts for only about one percent of the annual gelatin production due to the lower stability of the gel formed, poorer rheological properties than gelatin extracted from mammals, and allergic reactions [5Chicken foot gelatin has higher glycine, hydroxyproline, and proline content and higher thermal stability compared to mammalian and fish gelatin [6, 7Gelatin is a high molecular weight polypeptide. It is actually a derivative of collagen [8]. During thermal denaturation of collagen, structural changes and intramolecular bond breakage, chemical and physical changes occur, which ultimately lead to the production of gelatin. Gelatin properties such as gel strength and melting temperature are among the most important characteristics determining its commercial quality. These properties, in turn, are influenced by some factors such as molecular weight distribution and amino acid composition [9].

There are various methods for extracting gelatin from animal tissue cells, and the differences in these methods depend on the raw material. One of the best methods for extracting gelatin is the acid method, and the extracted gelatin has a desirable quality [10]. Acid treatment causes collagen to swell to increase the efficiency of gelatin extraction during thermal hydrolysis. The swelling and further solubilization of collagen is strongly influenced by the type and concentration of acid used [11]. This process removes organic substances such as blood, mucin, sugars, proteolectin, etc. (which are present in the raw material) [12]. Another method of producing gelatin is the microwave-assisted extraction technique.[2](MAE) is proposed as one of the most efficient methods to improve molecular and physicochemical properties such as gel strength, viscosity and melting point. The microwave device heats the material by radiating electromagnetic waves and causing water molecules and other polar molecules to move. These radiation waves are aligned one million times per second in the direction of the electric field and cause internal friction of the molecules, resulting in the generation of volumetric heat. Therefore, microwaves can be used to heat materials and extract them instantaneously [13This method can significantly reduce extraction time compared to conventional methods [14]. Reducing the cost and amount of solvent used are other advantages. Also, various microwave parameters such as the nature of the solvent, temperature, particle size, microwave power, and irradiation time affect the performance of the extracted material [15]. Therefore, in this study, two acid and microwave methods were used to extract gelatin from chicken feet, and the parameters of the acid extraction process, including time, temperature, concentration, and weight ratio of chicken foot paste to acid solution, were optimized, as were the parameters of gelatin extraction by microwave method with the simultaneous effect of power and time. Then, the properties of the optimized gelatin from both methods were compared with commercial bovine gelatin.

2- Materials and methods

2-1- Preparation of raw materials

In this study, chicken feet were purchased from a protein products store in Babol city. Chemicals such as sulfuric acid with a purity of 98%, sodium chloride, and all chemicals used in the study were purchased from Merck, Germany. Commercial bovine gelatin was also purchased from Kia Tejarat Batis Company.

2-2- Preparing the chicken legs

The purchased chicken feet were packed in nylon bags and transported to the laboratory. In the laboratory, the chicken feet were manually washed with water several times to remove blood and other excess materials. In the next step, the chicken feet were divided into smaller pieces by hand using a hand grinder and placed in a grinder to form a paste.

2-3- Extraction of gelatin by acid and microwave method

Gelatin extraction was performed based on the methods of Chaka et al. (2016) and Liu et al. (2019) with slight modifications [13, 16] For extraction, first the chicken leg paste was washed with warm water to reduce some of its fat. 100 g of chicken leg paste was mixed in 0.5 mol of sodium hydroxide solution in a weight/volume ratio of 2:1 using a mechanical mixer (Pars Azma, Iran) at a speed of 50 rpm for 10 minutes

1-Bovin Spongiform Encephalopathy

2-Microwave Assisted Extraction

to remove non-collagenous proteins. In the next step, the sample was washed with cold water until its pH was neutralized. After that, the sample was placed in a three-layer filter cloth to completely remove excess water. Then, the chicken leg paste was mixed in acid concentrations of 1 to 3 normal and the weight ratio of chicken leg paste to acid solution was 1:2-6 weight/volume using a mechanical mixer at a speed of 40 rpm for 60 minutes and the same as the previous steps, but this time until the pH reached 5.5 to 6, washing continued. Then, to extract gelatin, the sample was mixed with distilled water in a 3:1 weight/volume ratio and placed in a water bath (Memmert, WB14, Germany) and microwave (Techno, Te341/342, Germany) considering the variables of temperature, time and power. Then, the extracted solution was filtered with a three-layer filter cloth and poured into aluminum containers and dried for 24 hours in the acid method and 48 hours in the microwave method at a temperature of 45 degrees Celsius in a hot air oven (Memmert, ULM40, Germany). After the gelatin solution was dried, the obtained gelatin sheets were broken into smaller pieces and converted into powder by a grinder (Bosch, MKM6000, Germany) and passed through a sieve. The gelatin powder was stored in a refrigerator at 4 degrees Celsius and in polyethylene nylons until use for experiments.

2.4- Determining gelatin extraction efficiency

The gelatin extraction efficiency was determined in percentage based on the ratio of the weight of the gelatin powder obtained to the initial weight of the chicken leg paste using equation (1) [17].

Equation (1) 100×(initial weight of chicken leg paste/weight of dry gelatin powder) = gelatin extraction yield

2.5- Determining gel strength

Bloom value is the most important criterion for industrial gelatin grading. Strength is the strength of the gel, defined as weight per gram, and determines the final price. Different types of gelatin produce different gel strengths at different concentrations, causing changes in texture. Gel strength measurements of acid, microwave, and commercial gelatins were performed using a texture analyzer (Brookfield, Ct3-10, USA). First, a 67.6% gelatin solution was prepared, then it was placed in a refrigerator (temperature 5 to 7 degrees Celsius) for 16 hours before measuring gel strength. After the gel ripening time, the gel strength was determined using a cylindrical blancher with a probe diameter of 12.7 mm at a speed of 1 mm/s and a penetration value of 4 mm, and its bloom value (gel strength) was measured in grams [18].

2-6- TestSDS-PAGEChicken leg gelatin

This test is used to analyze protein patterns and determine the molecular weight of proteins. For this purpose, 5% separating gel and 6% consolidating gel were used to prepare polyacrylamide gel. Gelatin solution 10 mg/ml and 0.5 mol buffer mixture with pH = 6.8, 5% mercaptoethanol, 20% glycerol and 0.1% bromophenol aqueous were heated to 100°C for 5 minutes. After heating and cooling, the solution was injected into the wells of the consolidating gel and electrophoresis was performed at a speed of 15 mA [16].

2-7- Testing the browning rate of chicken leg gelatin

Transparency and color are important properties of gelatin and determine its application in various industries. Color parameters include L^* ، $a^*$and b^*The color of gelatin powder was measured using an IMG Pardazesh CAM-SystemXI colorimeter. The EΔ index represents the overall difference in measured color parameters between the standard sample and the test sample and can be used as a primary factor for evaluating color changes (Equation 2). Browning Index[3](BI) indicates the degree of product color change towards brown (Equation 3) [19].

Equation (2) $\Delta E = \sqrt{\Delta L^{*+2} + \Delta a^{*+2} + \Delta b^{*+2}}$

Equation (3) $BI = 100 \times \frac{(X-0.31)}{0.17}$

2-8- Viscosity test

Gelatin viscosity was measured using a standard method using a Brookfield DVII Ultra viscometer made in the United States. First, a 67.6% gelatin solution was prepared and the viscosity was measured in centipoise using a spindle at a speed of 100 rpm and a temperature of 40 degrees Celsius [17].

2-9- Optimization using the response surface method

Response surface method[4](RSM) is a set of mathematical and statistical techniques for modeling and analyzing problems in which one or more responses are affected by multiple variables. In this study, a central composite design was used.[5](CCD) was used. The central composite design is widely used to fit a quadratic model. Using this method, modeling will be possible and only requires a minimum number and number of experiments. In Tables 1 and 2, the levels of independent variables of the acid and microwave extraction process in the central composite design are shown in code. In this study, the optimization of gelatin extraction was carried out in the form of a fixed central composite design with 6 replications at the central point, in the form of 4-variable and 3-variable designs for the acid and microwave extraction methods, respectively. In this

3 -Browning index

4- Response Surface Methology

5 -Central Composite Design

design, 4 factors of acid concentration and weight ratio of chicken leg paste to acid solution, temperature and extraction time and 3 factors of acid concentration, power and microwave time were selected as independent variables and extraction efficiency and gel strength were selected as dependent variables, in which the input variables are indicated by the symbol X and the output response is indicated by the symbol Y.

Table 1- Range of experimental values of independent variables in the central composite design for optimizing the extraction of chicken feet gelatin by the acidic method

Independent variables	Levels		
Code	-1	0	1
Acid concentraion	1	2	3
Ratio of chicken feet to acid	2	4	6
Temperature	60	75	90
Time	1	3	5

Table 2- Range of experimental values of independent variables in the central composite design for optimizing the extraction of chicken feet gelatin by microwave method

Independent variables	Levels		
Code	-1	0	1
Acid concentraion	1	2	3
Microwave power	360	540	720
Time	10	15	20

These responses have been used for an empirical model and follow the quadratic equation pattern presented below for each of the acid methods in equation (4) and microwave in equation (5).

Equation (4) $Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_2^3 + b_{14}x_1x_2 + b_{24}x_1x_4 + e$

Equation (5) $Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_2^3 + b_{12}x_1x_2 + b_{23}x_2x_3 + e$

Polynomial coefficients with the symbol b_0 (constant coefficient), b_1، b_2، b_3 and b_4(linear effects), b_{11}، b_{22}، $b_{33}$and b_{44}(second-order effects) and b_{23}، b_{12}، $b_{14}$and b_{24}(interaction effects). In order to optimize the two methods of acid and microwave extraction of gelatin from chicken feet and analyze the two response factors of gelatin yield and gel strength, a central composite design was used using Design-Expert software version 2. 1. 11. Joint optimization of output factors was carried out to achieve the highest level of quality of produced gelatin, and finally, the T-TEST test was used to determine the difference between the predicted values and the actual values in the relevant responses, and the model was described through three-dimensional and two-dimensional graphs. The optimally extracted gelatin samples were analyzed by one-way analysis of variance using SPSS version 26 software in a completely randomized design, and the means were compared at a 5% error probability level with Duncan's multiple range test.

3- Results and discussion

3-1- Modeling the process of extracting chicken foot gelatin by acidic method

In the model of this research, the effect of 4 factors: acid concentration (normal), chicken foot weight ratio to acid solution (volumetric/weight), temperature (degrees Celsius), and time (hours) were selected as independent variables, and 2 factors: extraction efficiency (percentage) and gel strength (grams) were selected as dependent variables, in which the input variables were designated by the symbol X and the output response was designated by the symbol Y, and expressed by a quadratic polynomial equation as suggested by the software.

Equations (6) and (7), the extraction efficiency (Y_1) and gel strength (Y_2) as a function of concentration (X_1), temperature (X_2), extraction time (X_3) and the ratio of the weight of chicken feet to the acidic solution (X_4) is shown:

$AND_1 = -11.89 + 0.42X_1 + 0.28X_2 + 0.72X_3 - 0.39X_4 - 1.44X_1X_4 - 1.87X_1^2 - 3.49X_2^2 - 0.97X_3^2$ Equation (6)

$AND_2 = 203.42 + 9.61X_1 + 9.22X_2 + 6.56X_3 + 9.00X_4 + 6.75X_2X_4 - 25.68X_1^2 - 28.18X_2^2 - 21.18X_3^2$ Equation (7)

The quality and effectiveness of the presented model were determined through analysis of variance. According to the ANOVA table, the lack of fit test was not significant for both output factors ($P<0.05$). A good regression model is one whose test probability value (Lack of fit) is considered to be greater than the alpha significance level (usually five percent), meaning that the null hypothesis is accepted ($P<0.05$). Also, the X factors$_1$، X_2، X_3، X_4، X_1X_4، X_1^2، $X_2^2$and X_3^2 For the dependent variable extraction efficiency and X_1، X_2، X_3، X_4، X_2X_4، X_1^2، $X_2^2$and X_3^2 For the dependent variable gel strength at the significance level (95%), the coefficient of determination (R^2) for extraction efficiency and gel strength were obtained

as 0.98 and 0.95, respectively. The coefficient of determination is the proportion of variance in the dependent variable that can be predicted from the independent variables. This coefficient actually indicates the suitability of the model in predicting the output factors. Both R values^{2}The obtained coefficients of determination (R) were relatively high (close to 1), indicating a good fit between the experimental values and the predicted values from the models. The adjusted coefficient of determination $(R)^2$) for Y responses$_1$and Y_2The adjusted coefficient of determination, R value, was 0.96 and 0.91, respectively.^{2}The difference between R and R is adjusted according to the independent variables added to the regression line and the width of the new origin.^{2}and R^2The adjusted R value is less; it means that the independent variables added to the model were selected correctly.^{2}The predicted values for the extraction efficiency and gel strength responses were 0.93 and 0.85, respectively. This coefficient can be used to predict the model, and it is noteworthy that the value of this coefficient and the adjusted coefficient of determination should be close to each other and their difference should not be more than 0.2, so in the present research model, the differences are very small. Adeq precision measures the signal-to-noise ratio and indicates the applicability and suitability of the presented model in industrial processes. The value of this coefficient should be greater than 4, which in the model presented in this study is the dependent variable $Y_1$and Y_2were estimated to be 20.24 and 12.77, respectively, and the coefficient of variation (CV) for the extraction efficiency factor and gel strength were determined to be 6.85 and 8.23, respectively (Table 3). This coefficient is a measure of the distribution of statistical data and is obtained by dividing the standard deviation by the mean. In other words, the coefficient of variation expresses the amount of dispersion per unit of the mean. The lower the value of this coefficient (especially lower than 10), the better the equations. In the present study, the value of the coefficient of variation of both output variables was less than 10. Therefore, the presented model can be confirmed in both output responses.

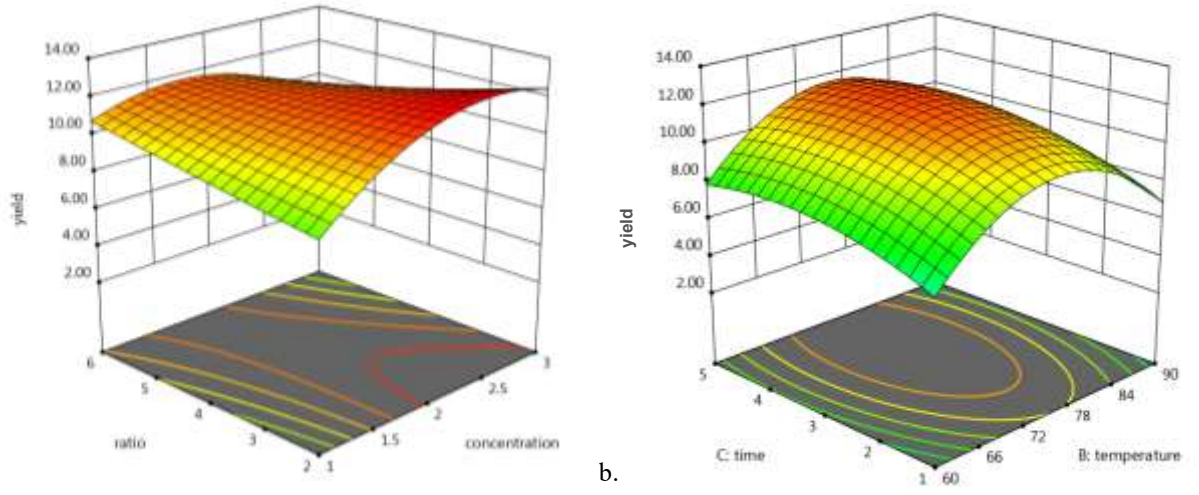
Table 3 - Analysis of Variance (ANOVA) regression for gel yield and strength responses by acidic extraction of chicken feet gelatin

		Yield				Strength			
Source	df	Coefficients	Sum of Squares	F-value	p-value	Coefficients	Sum of Squares	F-value	p-value
Model	14		289.41	66.85	<0.0001		49286.27	22.57	<0.0001
Linear coefficients									
b_1	1	0.42	3.32	10.73	0.0051	9.61	1662.72	10.66	0.0052
b_2	1	0.28	1.43	4.62	0.0484	9.22	1530.89	9.81	0.0068
b_3	1	0.72	9.40	30.41	<0.0001	6.56	773.56	4.96	0.0417
b_4	1	-0.39	2.78	8.98	0.0090	9.00	1458.00	9.35	0.0080
Quadratic coefficients									
b_{11}	1	-1.87	9.04	29.24	<0.0001	-25.68	1708.00	10.95	0.0048
b_{22}	1	-3.49	31.53	101.95	<0.0001	-28.18	2056.81	13.18	0.0025
b_{33}	1	-0.97	2.48	8.02	0.0126	-21.18	1161.76	7.45	0.0155
b_{44}	1	0.05	0.0069	0.0224	0.8829	-11.18	323.58	2.07	0.1704
Interaction coefficients									
b_{12}	1	-0.12	0.23	0.7685	0.3945	-0.50	4.00	0.0256	0.8749
b_{13}	1	0.04	0.03	0.1261	0.7274	1.75	49.00	0.3141	0.5835
b_{14}	1	-1.44	33.03	106.82	<0.0001	1.13	20.25	0.1298	0.7237
b_{23}	1	0.02	0.009	0.0307	0.8632	2.63	110.25	0.7067	0.4137
b_{24}	1	-0.18	0.54	1.76	0.2046	6.75	729.00	4.67	0.0472
b_{34}	1	-0.08	0.10	0.3363	0.5706	-4.00	256.00	1.64	0.2196
Residual	15		4.64				2340.03		
Lack of fit	10		3.18	1.09	0.4915		1680.69	1.27	0.4166
Error	5		1.46				659.33		
Total	29		294.04				51626.30		
R^2		0.98				0.95			
Adj-R^2		0.96				0.91			
Pr-R^2		0.93				0.85			
Precision Adeq		24.20				12.77			
CV		6.85				8.23			

3-2- Investigating the effect of independent variables on the extraction efficiency and gel strength of chicken foot gelatin

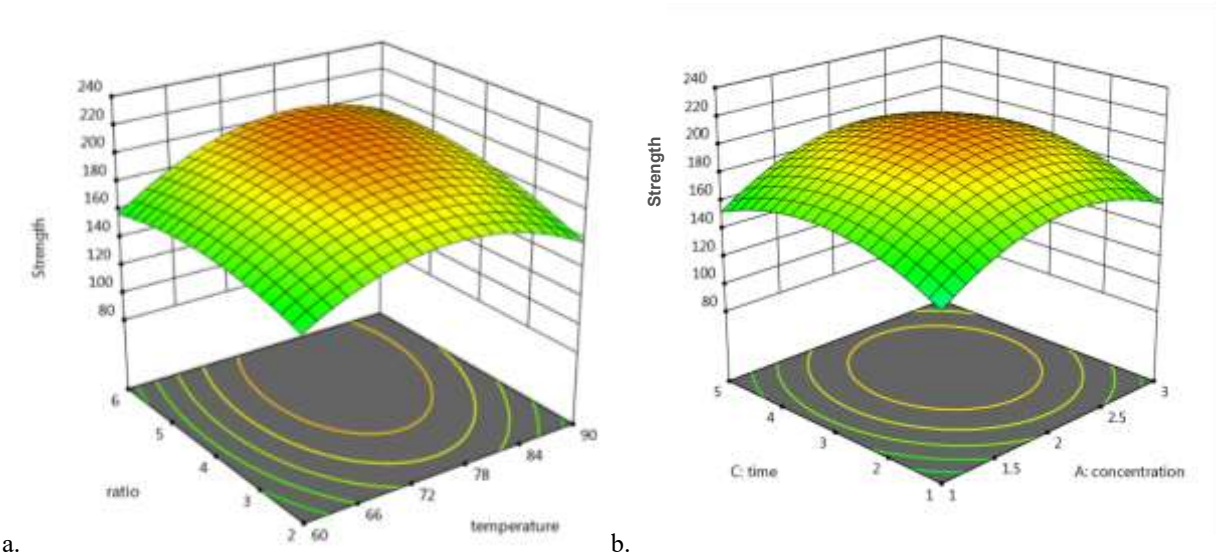
According to the results of Table 3, among the linear coefficients of the components of acid concentration and weight ratio of chicken leg paste to acid solution, temperature and extraction time had significant effects on the yield and strength of gelatin extraction gel, of which time and concentration had the greatest effect on gelatin extraction efficiency. Among the second-order components, temperature and acid concentration, and among the interaction effects, the component of concentration-weight ratio of chicken leg to acid solution had the greatest effect on the extraction efficiency. Figure 1 shows the three-dimensional response surfaces of the combined effect of concentration-weight ratio of chicken leg to acid solution and temperature-time on extraction efficiency. With increasing acid concentration at high chicken leg to acid ratio or with increasing chicken leg to acid ratio at high acid concentration and also increasing the process temperature, the extraction efficiency decreased sharply. In fact, increasing temperature and acid volume, respectively, causes the evaporation of gelatin solution and destruction of chicken leg collagen, and as a result, destroys its protein structure and reduces gelatin weight.

Among the interaction effects, temperature-chicken foot weight ratio to acid showed the highest significant effect on gelatin gel strength. According to Figure 2, with excessive increase in ratio and temperature, gelatin gel strength decreased, protein structures weakened, and the trend became downward.



a. b.

Figure 1- Three-dimensional response of the acidic process of chicken feet gelatin extraction: a) The effect of acid concentration and the ratio of chicken feet to acid, b) The effect of temperature and time on the extraction yield



a. b.

Figure 2- Three-dimensional response of the acidic process of chicken feet gelatin extraction: a) The effect of acid concentration and the ratio of chicken feet to acid, b) The effect of temperature and time on gel strength

3-3- Joint optimization of the chicken foot gelatin extraction process using the acidic method

One of the main objectives of this study was to find the optimal parameters for maximum gelatin production. Therefore, the extracted gelatin should have high yield as well as desirable gel strength for use in industrial processes. Table 4 shows the predicted and actual values with relatively small errors in three replicates for gelatin extraction yield and gel strength. The calculated data indicate that these values are in good agreement, confirming the accuracy of the models.

Table 4- The predicted and experimental values of the dependent variable for the optimal sample of chicken feet gelatin extracted by the acidic method

Response	Experimental data	Predicted data
Yield	11.44[a]	11.89[a]
Gel strength	206[a]	203[a]

Based on the optimization of the acid method, a temperature of 76.40°C, a ratio of 1:4 (volume/weight) of chicken leg weight to acid solution, a concentration of 2 normal acid, and an extraction time of 3 hours were determined as the optimum point in this method. In this study, the gelatin extraction efficiency varied from 3.10 to 12.65. The gelatin yield depends on the pretreatment process in addition to the raw materials. Sarbon et al. (2015) extracted gelatin from chicken skin using alkaline pretreatment followed by acid and achieved only a yield of 16.2% [12]. Ma et al. (2018) reported that the extraction yield of pig bone gelatin using the hydrochloric acid pretreatment method was only about 3% [20]. Hao et al. (2009) in a study on sturgeon skin pretreated with calcium hydroxide increased the yield of gelatin from 2.40 to 3.52% [21The yield of gelatin extracted from red fish was 81.7%, black tilapia was 39.5%, shortfin scad was 25.7%, and chicken skin was 16.2% [22]. The extraction efficiency of gelatin from chicken feet was reported by Chiu et al. (2018) using hydrochloric acid at different temperatures ranging from 4.65 to 5.31 percent [23]. The efficiency of gelatin extraction is strongly related to the stability of the collagen structure. In general, acid pretreatment destabilizes cross-links and leads to the destruction of the regular structure of natural collagen, thereby facilitating gelatin extraction [24]. The research results clearly show that the standardized extraction process in our study has a relatively higher yield of chicken foot gelatin (12.65%). Cao et al. (2020) investigated the percentage effects of different acids and pepsin on the properties of gelatins extracted from bovine bone collagen [25In this study, the yield of bovine bone gelatin ranged from 4.26 to 11.75 percent, which is similar to the present study.

The gel strength values obtained in this study ranged from 96 to 223 g. The gel strength of bovine gelatin (200 g) was lower than that of the optimized chicken leg gelatin (206 g). Gel strength is strongly dependent on the amount of alpha chains in the gelatin. Since a high proportion of peptides with molecular weight lower than alpha chains can reduce gel strength, the higher the alpha chains in the gelatin template, the higher the gel strength. The researchers also reported that the stability of the triple helix structure in the reconstituted gelatins is proportional to the total content of pyrrolidine imino acids. Hydroxyproline is believed to play a unique role in stabilizing the collagen triple helix due to its hydrogen bonding ability [9]. Aikin-Dincer et al. (2017) demonstrated that broiler chicken skin had a gel strength of 166 g. Chicken leg skin and tendon gelatin at a concentration of 33.3% had a lower gel strength than gelatin at a concentration of 67.6%. Thus, as expected, gel strength increased with gelatin concentration [26]. Sai-Leo et al. (2016) determined the gel strength of gelatin extracted from sea bass skin with different pretreatments and defatting processes at 219, 202 and 222 g [27]. The difference in gel strength between samples could be due to differences in intrinsic properties such as molecular weight distribution, protein chain length, and complex interactions determined by the amino acid composition and the ratio of alpha/beta chains present in the gelatin [28The gel strength of collagen extracted from chicken feet using different acids such as acetic, lactic and citric acids at different concentrations ranged from 123 to 204 g, and the gel strength of gelatin extracted using 1.5% v/v acetic acid showed the highest Bloom value (204 g) [22].

The gel strength of non-mammalian gelatins such as fish gelatin (mackerel 180 g, tilapia 181 g, sea croaker and shortfin 125 and 177 g) was determined by Badiei and Hovel (2006) [28] which is lower than that of chicken leg gelatin (acidic) in this study. The low hydroxyproline content of fish skin gelatin was one of the main reasons for the low gel strength of gelatin. A higher bloom value contributes to a higher melting and gelling point and a shorter gelling time of the final product. In addition, the bloom strength of gelatin also depends on factors such as acid concentration, soaking time and extraction temperature. In general, gelatin with high bloom contributes to better foaming and emulsifying properties of the final product compared to gelatin with relatively low bloom [9].

3-4- Modeling the process of extracting chicken leg gelatin using microwave method

From the results obtained from the measurement of target factors, including extraction efficiency (Y_1) and gel strength (Y_2), at different levels of independent variables including sulfuric acid concentration (X_1), power (X_2) and extraction time (X_3) According to the software suggestion, the quadratic model was selected for the extraction efficiency and gel strength factors.

$AND_1 = 10.26 + 1.38X_1 + 2.11X_2 + 1.34X_3 + 0.39X_1X_2 + 0.13X_1X_3 - 0.66X_2X_3 - 2.44X_1^2 + 1.80X_3^2$Equation (8)

$AND_2 = 203.42 + 9.61X_1 + 9.22X_2 + 6.56X_3 - 25.68X_1^2 - 28.18X_2^2$Equation (9)

The quality and effectiveness of the presented model were determined through analysis of variance. According to Table 5, the lack of fit test for both output factors $Y_1$and $Y_2$was not significant (P<0.05). Also, the constant coefficient and X factors$_1$، X_2، X_3، X_1X_2، X_1X_3، X_2X_3، $X_1^2$and X_3^2For the dependent variable, extraction efficiency and constant coefficient and X factors$_1$، X_2، X_3، X_1^2، X_2^2For the dependent variable, gel strength, it was significant at the 95% level. The coefficient of determination R^2The responses for extraction efficiency and gel strength were 0.99 and 0.98, respectively, so both R values were^{2}The obtained coefficients were relatively high (close to 1), indicating a good fit between the experimental values and those predicted by the models. The adjusted coefficient of determination for the responses $Y_1$and Y_2They were 0.98 and 0.97, respectively, and there was a very small difference with the predicted R value.^{2}(For Y variables$_1$ and Y_2(0.97 and 0.93, respectively). Also, the signal-to-noise ratio for extraction efficiency and gel strength was 50.53 and 26.77, respectively, and the coefficient of variation for efficiency was 3.41% and for gel strength was 3.53%. Therefore, the proposed model is favorable.

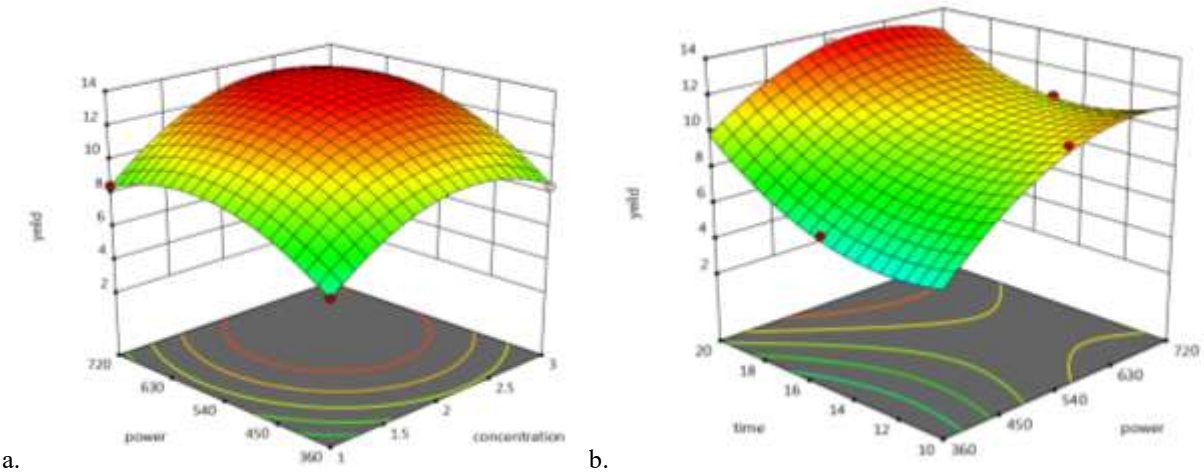
Table 5 - Analysis of Variance (ANOVA) regression for gel yield and strength responses by microwave extraction of chicken feet gelatin

		Yield				Strength			
Source	df	Coefficients	Sum of Squares	F-value	p-value	Coefficients	Sum of Squares	F-value	p-value
Model	9		137.67	164.39	<0.0001		42731.90	97.09	<0.0001
Linear coefficients									
b_1	1	1.38	19.15	208.88	<0.0001	9.61	883.60	18.07	0.0017
b_2	1	2.11	44.35	483.66	<0.0001	9.22	324.90	6.64	0.0275
b_3	1	1.34	17.88	194.93	<0.0001	6.56	302.50	6.19	0.0322
Quadratic coefficients									
b_{11}	1	-2.44	16.34	178.21	0.0043	-25.68	3885.96	79.49	<0.0001
b_{22}	1	-2.09	11.99	130.71	0.2280	-28.18	11651.27	238.24	<0.0001
b_{33}	1	1.80	8.88	96.87	0.0001	-21.18	23.27	0.47	0.5060
Interaction coefficients									
b_{12}	1	0.39	1.23	13.44	<0.0001	-0.50	28.13	0.57	0.4657
b_{13}	1	0.13	0.15	1.65	<0.0001	1.75	6.13	0.12	0.7308
b_{23}	1	-0.66	3.51	38.29	<0.0001	1.13	6.13	0.12	0.7308
Residual	10								
Lack of fit	5			1.99	0.2336			2.66	0.1531
Error	5								
Total	19								
R^2		0.99				0.98			
Adj-R^2		0.98				0.97			
Pr-R^2		0.97				0.93			
Precision Adeq		50.53				26.77			
CV		3.41				3.53			

3-5- Investigating the effect of independent variables on gelatin extraction efficiency

According to the results of Table 5, among the linear coefficients, microwave power and acid concentration had the greatest effect on gelatin yield. Among the quadratic components, acid concentration had the greatest effect, and among the interaction effects, power-time and concentration-power components had the greatest effect on the yield. Figure 3 shows the response surfaces of the combined effect of concentration-power and time-power. In this figure, it was found that with increasing power to 720 W, due to the loss of protein structure and evaporation of the amount of gelatin solution, the yield curve becomes downward. Also, with increasing time (at constant power), the graph assumed an upward trend. According to Figure 3, at low values of time and power, a strong decreasing effect on gelatin yield was

observed. According to Table 5, the highest coefficients and F-value were related to power and concentration. Therefore, concentration and power are two very significant factors in this extraction method.



a. b.

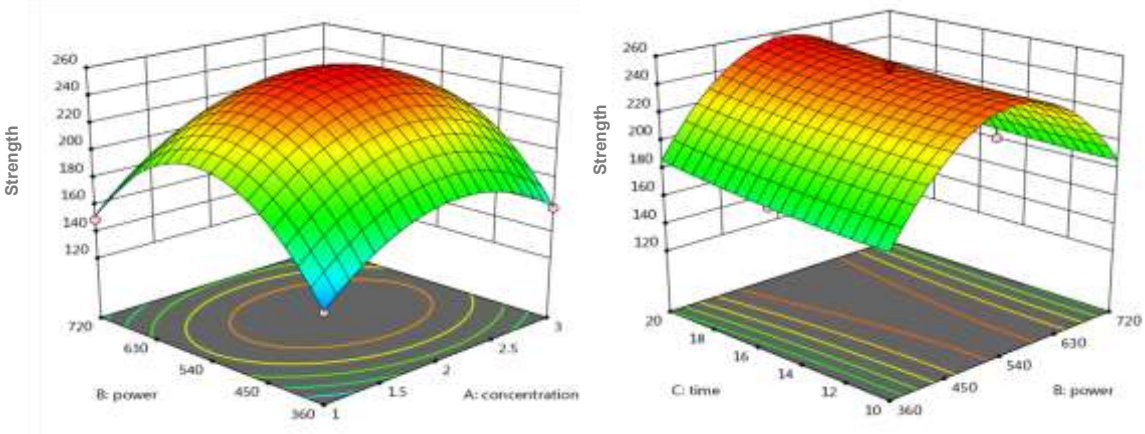
Figure 3 - Combined effect of concentration-power and power-time on the gelatin extraction yield by microwave method

3-6- Investigating the effect of independent variables on gelatin gel strength

According to Table 5, all three factors of acid concentration, extraction power and time as well as the quadratic effects of concentration and power were considered significant in this model. Concentration with F value of 18.07 had the greatest effect on gel strength. Also, power and time with F values of 6.64 and 6.19, respectively, had similar effects on gelatin gel strength. The quadratic factors of power and concentration also had F values of 238.24 and 79.49, respectively.

exerted significant effects on gel strength. None of the interaction components had significant effects on gel strength. With

Referring to Figure 4, with excessive increase in concentration and power, the strength of the gelatin gel decreased, which can be attributed to the weakening of protein structures.



a. b.

Figure 4 - Combined effect of concentration-power and power-time on the gelatin extraction yield by microwave method

3-7- Joint optimization of the chicken foot gelatin extraction process using microwave method

*Based on software optimization, optimal conditions for gelatin extraction in the microwave method Includes 2 normal acids, 20 minutes of time, and 540 watts of microwave power.*Table 6 Predicted and actual values (with relatively small errors, in three replicates) for gelatin extraction yield and gel strength in the method*Microwave*The presented results indicate that there is very little difference between the predicted and tested values. Therefore, the presented model can be verified.

Table 6- The predicted and experimental values of the dependent variable for the optimal sample of chicken feet gelatin extracted by the microwave method

Response	Experimental data	Predicted data
Yield	10.48[a]	10.25[a]
Gel strength	253[a]	247[a]

Extraction efficiency is certainly an important factor to consider when determining the optimal conditions for industrial gelatin production because it can significantly affect the feasibility of mass production of gelatin. The heat treatment associated with any of the different extraction methods breaks the hydrogen bonds in the collagen molecule, thus irreversibly disrupting the three-dimensional structure and leading to the dissolution of collagen to form gelatin [29The extraction method can significantly affect the yield of gelatin [14]. In the microwave extraction method of chicken leg gelatin, the yield of gelatin ranged from 2.10 to 13.37%. Liu et al. (2019) investigated the yield, structure, and properties of rabbit skin gelatin using microwave. Compared with gelatin extracted with a water bath, gelatin extracted using the microwave method was able to have a significant yield in a short time (5 to 30 minutes). The yield of gelatin extracted by microwave increased with increasing extraction time, indicating the positive effect of microwave. With increasing microwave time from 5 to 60 minutes, the yield reached 44.6%, and then with further increasing microwave extraction time from 60 to 90 minutes, the yield decreased slightly [13In published articles, the yield of gelatin extracted by microwave is relatively low, with values of 0.75, 1.27, and 0.91% reported [30]. In this study, the yield was up to 13.37%, which is higher than other studies. In the study of Park et al. (2013), during the extraction of gelatin by three methods: microwave, superheated steam, and water bath, the average yield of gelatin obtained was 0.75%, which is lower than the present study [14]. Kim et al. (2020) investigated the optimal conditions for swelling duck skin and methods for extracting gelatin from it as a new source. Gelatin was extracted using water bath, ultrasound, superheated steam, and microwave methods. In this study, the yield of microwave-extracted gelatin was reported to be 2.05% [31] which was lower than the present study.

The gel strength of chicken leg gelatin obtained by microwave extraction was higher than that of commercial bovine gelatin (200 g). In the study of Liu et al. (2019) who extracted rabbit skin gelatin by microwave, the gel strength was reported to be up to 400 g. Gelatins obtained by microwave extraction at times of 5, 15, and 30 min showed significant gel strength compared to water bath, indicating that short microwave extraction time can achieve better gel strength. It is possible that the short microwave extraction time can properly release its subunit components to achieve higher gel strength. As the microwave extraction time was prolonged from 60 to 90 min, the gel strength decreased significantly. The decrease in gelatin strength may be due to the decrease in amino acid content or the destruction of high molecular weight subunits in gelatin [13]. In the study by Kim et al. (2020), the gel strength of gelatin extracted from duck skin using the water bath and microwave extraction method was higher compared to other methods [31]. The results from Park et al. (2013) showed that microwave showed the highest gel strength because the short process time in microwave extraction resulted in collagen extraction before the protein was completely denatured. The gel strength of gelatin obtained from microwave extraction in this study was reported to be 260 g [14] which is similar to the present study. Fanj et al. (2021) investigated microwave irradiation as a new process for improving the physicochemical properties of pig skin gelatin and determined that with increasing microwave irradiation time, the gel strength gradually decreased so that the gel strength reached the lowest value of 460 g at 30 minutes [32] which is more than the present study.

3-8- Comparison*Chicken foot gelatin extracted by acid and microwave methods compared to commercial gelatin*

3-8-1- SDS-PAGE examination

SDS-PAGE is the most commonly used analytical method for the identification of collagen chains. Gelatin is a mixture of collagen fragments obtained by hydrolysis of collagen amide bonds in the main chain of collagen molecules with molecular weights in the range of 16 to 150 kDa [33The molecular weight distribution, structure, and composition of subunits, including alpha and beta chains and low molecular weight protein fragments, have a significant impact on the functional and physical properties of gelatins [34]. In fact, electrophoretic analysis helps to identify protein integrity and recover different gelatin components (due to variations in extraction conditions) [35]. The SDS-PAGE profiles of gelatins from chicken feet and commercial bovine gelatin are shown in Figure 5. For all three types of gelatin, alpha-1 and alpha-2 chains were observed. According to Figure 5, in the optimized sample of microwave gelatin, brighter bands were observed and accounted for a higher percentage of the molecular weight, and this parameter greatly affected the yield and strength of the final product. The heating of materials by microwave is mainly due to the dipolar nature of water. When an oscillating electric field hits water molecules, the dipolar molecules are constantly (one

million times per second) aligned in the direction of the electric field, which causes internal friction of the molecules and consequently volumetric heating. During the microwave extraction process, the vibrational force of water molecules, together with the thermal energy, causes the instantaneous cleavage of covalent bonds and consequently the dissolution of collagen. Therefore, high molecular weight subunits in microwave extracted gelatin can be rapidly dissolved. But on the other hand, they can be completely destroyed with increasing microwave time [15].

In the SDS-PAGE profiles of gelatins extracted from chicken and turkey heads at 50 and 60°C, alpha-1 and alpha-2 chains were also observed, which were similar to the optimized chicken leg gelatin sample in this study. It seems that turkey head gelatin had a higher number of high molecular weight bands and also more bands in the lower molecular weight range than chicken head gelatin. In addition, it seems that gelatin degradation occurs with increasing preparation temperature, and more low molecular weight segments are formed. According to Moyang et al. (2004), high temperature increases the cleavage of gelatin chains, resulting in the formation of lower molecular weight components [36]. In general, gelatin with higher alpha chain content showed better functional properties (including gelling, emulsifying, and foaming properties) [37]. The results of the molecular weight distribution of gelatins after pretreatment of whiptail skin with hydrochloric acid and acetic acid at different concentrations showed that regardless of the type of acid used, the electrophoretic profile of gelatin consisted of two protein bands, beta and alpha (with molecular weights of 200 and 125 kDa) and one band with a density of less than 100 kDa. Hydrochloric acid enhanced the hydrolysis of collagen and presented protein bands with various molecular weights (57-84 kDa). However, acetic acid produced bands with a value of 125 and 100 kDa (alpha one and alpha two, respectively) that were denser than hydrochloric acid, indicating its advantage for protein extraction. Therefore, the extraction of whiptail skin gelatin depended on the concentration of the acid used [38] which is somewhat similar to the optimized gelatin sample (acidic and microwave) in the present study. Cao et al. (2020) investigated the molecular weight of different gelatins extracted from bovine bone collagen [25]. The results of quantitative analysis of alpha-1 and alpha-2 chains in gelatin samples showed that the higher band intensity of collagen with molecular weight (70-100 kDa and 15-25 kDa) in acid pre-treatment and post-treatment was lower than the optimal chicken foot gelatin sample in this study. In fact, alpha-chain in gelatin is well correlated with the functional properties of gelatin including its strength with bloom degree, viscosity and low melting point [17]. Kanwat et al. (2017) showed that pretreatment with high acid concentration leads to excessive degradation of collagen molecules and a decrease in the amount of beta chains and fragments with a molecular weight of less than 200 kDa. They also reported the protein patterns of gelatins extracted from rohu fish using different acids with molecular weights of 32, 40 and 80 kDa. The results showed that different acids on rohu fish cause excessive degradation of collagen molecules, which ultimately leads to the removal of protein molecules (gelatin) during washing with water [39].

The results of SDS-PAGE protein quantification of samples extracted from duck skin using different extraction methods by Kim et al. (2020) showed that samples obtained from superheated steam (24.61 µg/mL) and microwave (97.59 µg/mL) had higher protein concentrations than samples obtained from the water bath method (19.46 µg/mL) and ultrasonic extraction methods [31]. Also, the samples obtained from these two methods had more intense bands than the samples obtained from the water bath and ultrasound extraction methods, indicating more gelatin degradation and was consistent with the results of this study. Protein patterns of gelatin extracted from goat skin at different temperatures by Mad Ali et al. (2017) for different times showed that alpha chains were the main components in all gelatin samples. Beta chains were also found in gelatins extracted at temperatures of 50 and 60 degrees Celsius; however, beta chains were not found in samples extracted at 70 degrees Celsius, regardless of the extraction time. Alpha one and alpha two chains had molecular weights of 131 and 124 kDa, respectively. The results showed that the degradation of alpha and beta chains was evident with increasing temperature and extraction time [31].

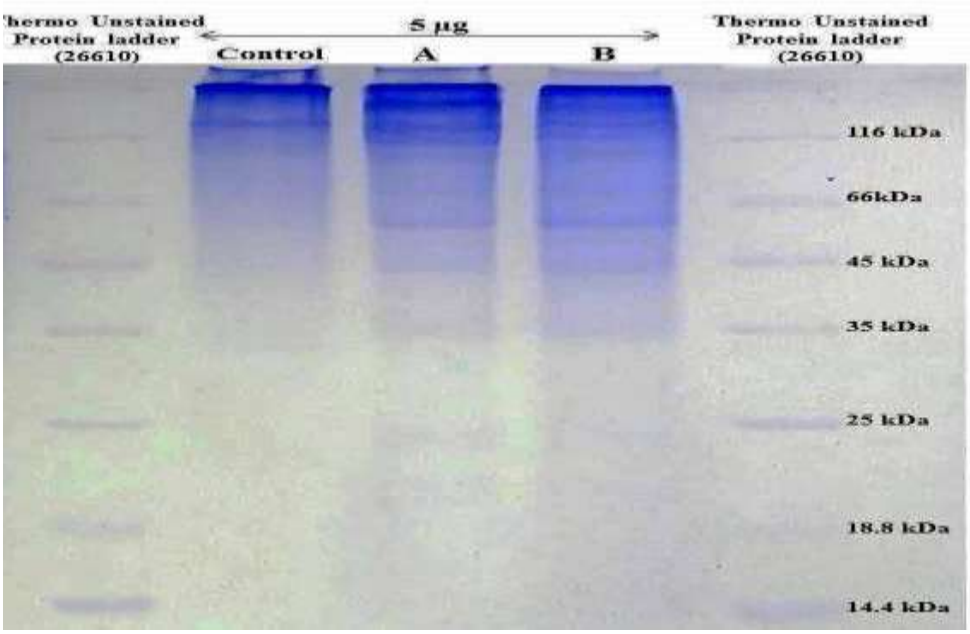
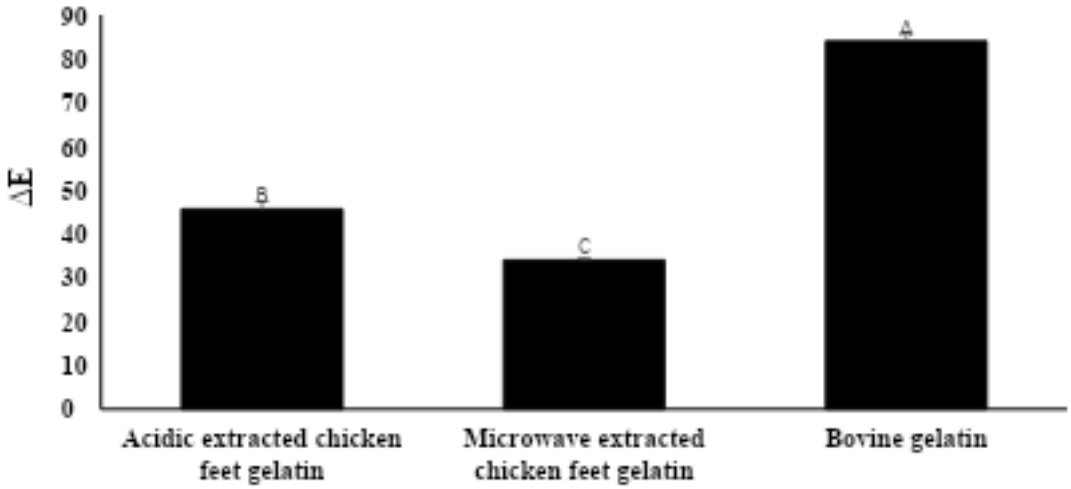


Figure 5 – The SDS-PAGE electrophoretic pattern of the optimal chicken feet gelatin of acidic extraction (B), microwave extraction (A) and commercial bovine gelatin (Control)

3-8-2- Colorimetry

The color of gelatin is significantly affected by raw materials, extraction process, and drying, resulting in differences in color and consumer acceptance [40]. The drying method may have a great influence on the color of gelatin [41].



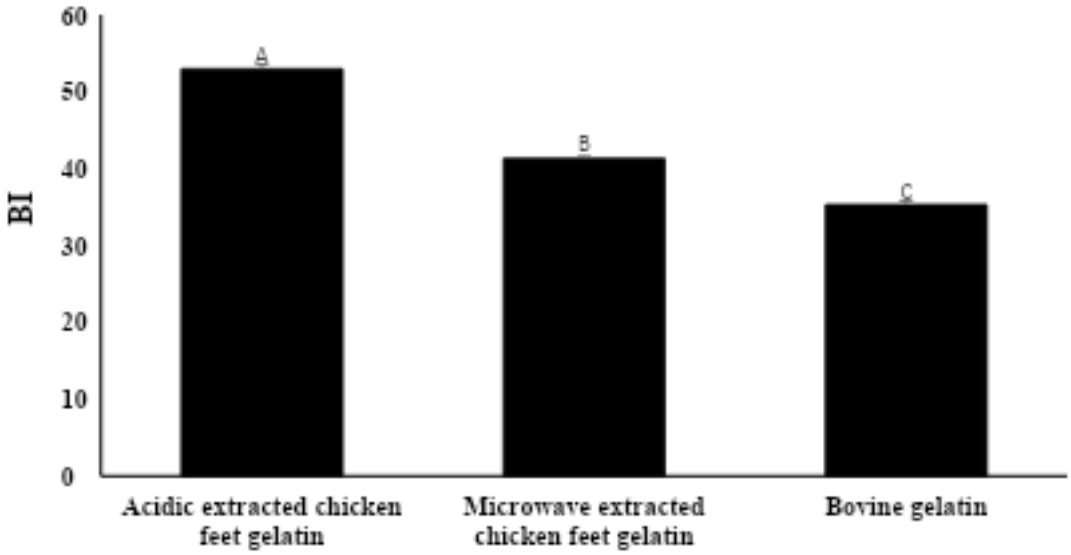


Figure 6- Color changes (ΔE) and browning index (BI) of microwave and acidic extracted chicken feet gelation compared to commercial bovine gelatin

The highest EΔ value was found in the bovine gelatin sample. The highest browning index was found in the acid gelatin sample (Figure 6). Non-enzymatic browning occurs from the reaction between the free amine groups of the protein with the glucosidic hydroxyl groups of reducing sugars or carbonyl compounds such as aldehydes and ketones. During the roasting process, the amount of amine and hydroxyl compounds increases, thus the rate of non-enzymatic browning reactions increases.

Rahim et al. (2021) investigated gelatins extracted from chicken feet and heads. Results of color factors b*، a*and L*The results of the two mentioned samples were reported as 17.93, 0.17, and 53 for chicken feet and 15.30, 1.73, and 62 for chicken heads, respectively [42] which is similar to the results of the color factors obtained in the present study. In another study, gelatin was extracted from duck skin using different methods. The highest brightness value of 42.56 was obtained for gelatin extracted by ultrasound, while the lowest brightness value was obtained for gelatin extracted by superheated steam. After the ultrasound extraction method, the highest brightness value of 28 was obtained for microwave extraction. It should be noted that the redness and yellowness of gelatin extracted by different thermal methods showed statistical differences [31Vidyasari et al. (2014) measured the color of chicken leg gelatin using acid extraction method at values of b=0.34.*، 24/8=a*and 45/61=L* They reported [43Park et al. (2013) examined gelatin extracted from duck feet using three different methods, and among them, the color of gelatin extracted by microwave method with b value*، a*and L*1.19, 0.99, 34 were reported respectively [14According to Rahman and Jamalia (2012), the brightness values of chicken foot gelatin (24/42) were significantly higher than commercial bovine gelatin (36/40).44]. Al et al. (2020) extracted gelatin from camel skin at three different ages of 2.5, 4.5 and 7 years by heating and chemical pretreatment. The color of the extracted gelatin did not show significant differences among the samples. However, the gelatin powder had a golden yellow color. All samples had valuesLighting 12/28 to 02/41and showed greater brightness and less yellowness than commercial bovine gelatin [45Almedia et al. (2012) investigated the physicochemical properties of gelatin extracted from chicken leg skin and tendon as a substitute for mammalian gelatin (which is commonly used commercially) and the color of the gelatin powder obtained with b values*، a*and L*They reported on 2/19, 3/01 and 3/51 respectively [46]. As a result, the brightness value of the optimal chicken leg gelatin sample in this study is higher than the studies mentioned. Park et al. (2013) reported that the use of microwaves increased brightness and had a significant difference with other methods. In addition, skin pretreatment with this method led to an increase in yellowness values. It is believed that this is related to the production of free amino groups in the resulting gelatin [14].

4- Conclusion

According to the results obtained from the experiments in this study, using the variables of acid concentration (2 normal), chicken foot paste to acid solution ratio (1 to 4 volume/weight), temperature (76.40 degrees Celsius) and extraction time (3 hours), in the acid method, gelatin can be obtained with a maximum yield of 89.11% and a gel strength of 203 grams. Also, in the concentration (2 normal), power (540 watts) and time (20 minutes), a gel strength of 247 grams and a yield of more than 10 grams can be obtained. Therefore, both the acid and microwave extraction processes can be modeled by the response surface regression method. Based on the results of

comparison with commercial gelatin, it was proven that gelatin extracted from chicken feet has superior properties and can potentially be used as an alternative source of mammalian gelatin.

5-Resources

[1] Lasekan, A., Bakar, F. A., & Hashim, D. (2013). Potential of chicken by-products as sources of useful biological resources. *Waste management*, *33*(3), 552-565.

[2] Fatima, S., Mir, M. I., Khan, M. R., Sayyed, R. Z., Mehnaz, S., Abbas, S., ... & Masih, R. (2022). The optimization of gelatin extraction from chicken feet and the development of gelatin based active packaging for the shelf-life extension of fresh grapes. *Sustainability*, *14*(13), 7881.

[3] Mokrejš, P., Mrázek, P., Gál, R., & Pavlačková, J. (2019). Biotechnological preparation of gelatines from chicken feet.*Polymers*, *11*(6), 1060.

[4] Kaewdang, O., Benjakul, S., Kaewmanee, T., & Kishimura, H. (2014). Characteristics of collagens from the swim bladders of yellowfin tuna (Thunnus albacares). *Food Chemistry*, *155*, 264-270.

[5] Shahidi, F., & Botta, J. R. (2012).*Seafoods: chemistry, processing technology and quality*. Springer Science & Business Media.

[6] Nik Aisyah, N. M., Nurul, H., Azhar, M. E., & Fazilah, A. (2014). Poultry as an alternative source of gelatin. *Health and the Environment Journal*, *5*(1), 37-49.

[7] Bichukale, A. D., Koli, J. M., Sonavane, A. E., Vishwasrao, V. V., Pujari, K. H., & Shingare, P. E. (2018). Functional properties of gelatin extracted from poultry skin and bone waste. *International Journal of Pure & Applied Bioscience*, *6*(4), 87-101.

[8] Gál, R., Mokrejš, P., Mrázek, P., Pavlačková, J., Janáčová, D., & Orsavová, J. (2020). Chicken heads as a promising by-product for preparation of food gelatins.*Molecules*, *25*(3), 494.

[9] Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. A., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food hydrocolloids*, *25*(8), 1813-1827.

[10] Abedinia , A. , Nafchi , A. M. , Sharifi , M. , Ghalambor , P. , Oladzadabbasabadi , N. , Ariffin , F. , & Huda , N. (2020). Poultry gelatin: Characteristics, developments, challenges, and future outlooks as a sustainable alternative to mammalian gelatin. *Trends in Food Science & Technology*, *104*, 14-26.

[11] Damrongsakkul, S., Ratanathammapan, K., Komolpis, K., & Tanthapanichakoon, W. (2008). Enzymatic hydrolysis of rawhide using papain and neutrase. *Journal of industrial and Engineering Chemistry*, *14*(2), 202-206.

[12] Sarbon, N. M., Badii, F., & Howell, N. K. (2015). The effect of chicken skin gelatin and whey protein interactions on rheological and thermal properties. *Food Hydrocolloids*, *45*, 83-92.

[13] Liu, T., Dai, H., Ma, L., Yu, Y., Tang, M., Li, Y., ... & Zhang, Y. (2019). Structure of Hyla rabbit skin gelatin as affected by microwave-assisted extraction. *International journal of food properties*, *22*(1), 1594-1607.

[14] Park, J. H., Choe, J. H., Kim, H. W., Hwang, K. E., Song, D. H., Yeo, E. J., ... & Kim, C. J. (2013). Effects of various extraction methods on quality characteristics of duck feet gelatin. *Food Science of Animal Resources*, *33*(2), 162-169.

[15] Moret, S., Conchione, C., Srbinovska, A., & Lucci, P. (2019). Microwave-based technique for fast and reliable extraction of organic contaminants from food, with a special focus on hydrocarbon contaminants. *Foods*, *8*(10), 503.

[16] Sabzi, F., Varidi, M. J., Varidi, M., & Asnaashari, M. (2024). Effect of verjuice (Vitis vinifera L.) on physicochemical and textural properties of beef M. biceps femoris. *Food Science & Nutrition*, *12*(8), 5497-5517.

[17] Erge, A., & Zorba, Ö. (2018). Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment. *Lwt*, *97*, 205-212.

[18] Farahmandfar, R., Asnaashari, M., Amraie, M., & Salehi, M. (2019). Color and weight changes of fresh-cut banana slices coated by quince seed gum: Effect of concentration, storage temperature and duration. *Iranian Food Science and Technology Research Journal*, *14*(6), 75-85.

[19] Farahmandfar, R., Mohseni, M., & Asnaashari, M. (2017). Effects of quince seed, almond, and tragacanth gum coating on the banana slices properties during the process of hot air drying. *Food science & nutrition*, *5*(6), 1057-1064.

[20] Ma, M., Ma, L., Yu, W., Zhang, X., Shen, Y., & Zhang, Y. (2018). Research on rapid gelatinization of rabbit skin collagen as effect of acid treatment. *Food Hydrocolloids*, *77*, 945-951.

[21] Hao, S., Li, L., Yang, X., Cen, J., Shi, H., Bo, Q., & He, J. (2009). The characteristics of gelatin extracted from sturgeon (Acipenser baeri) skin using various pretreatments. *Food Chemistry*, *115*(1), 124-128.

[22] Chakka, A. K., Muhammed, A., Sakhare, P. Z., & Bhaskar, N. (2017). 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*, *8*, 2583-2593.

[23] Choe, J., & Kim, H. Y. (2018). Effects of chicken feet gelatin extracted at different temperatures and wheat fiber with different particle sizes on the physicochemical properties

of gels. *Poultry Science*, *97*(3), 1082-1088.

[24] Benjakul, S., Thiansilakul, Y., Visessanguan, W., Roytrakul, S., Kishimura, H., Prodpran, T., & Meesane, J. (2010). Extraction and characterisation of pepsin-solubilised collagens from the skin of bigeye snapper (Priacanthus tayenus and Priacanthus macracanthus). *Journal of the Science of Food and Agriculture*, *90*(1), 132-138.

[25] Cao, S., Wang, Y., Xing, L., Zhang, W., & Zhou, G. (2020). Structure and physical properties of gelatin from bovine bone collagen influenced by acid pretreatment and pepsin. *Food and Bioproducts Processing*, *121*, 213-223.

[26] Aykın-Dinçer, E., Koç, A., & Erbaş, M. U. S. T. A. F. A. (2017). Extraction and physicochemical characterization of broiler (Gallus gallus domesticus) skin gelatin compared to commercial bovine gelatin. *Poultry Science*, *96*(11), 4124-4131.

[27] Sae-Leaw, T., Benjakul, S., & O'Brien, N. M. (2016). Effect of pretreatments and defatting of seabass skins on properties and fishy odor of gelatin. *Journal of Food Biochemistry*, *40*(6), 741-753.

[28] Badii, F., & Howell, N. K. (2006). Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. *Food hydrocolloids*, *20*(5), 630-640.

[29] Ahmad, M., & Benjakul, S. (2011). Characteristics of gelatin from the skin of unicorn leatherjacket (Aluterus monoceros) as influenced by acid pretreatment and extraction time. *Food Hydrocolloids*, *25*(3), 381-388.

[30] Li, S., Zeng, W., Li, R., Hoffman, L. C., He, Z., Sun, Q., & Li, H. (2018). Rabbit meat production and processing in China. *Meat science*, *145*, 320-328.

[31] Kim, T. K., Ham, Y. K., Shin, D. M., Kim, H. W., Jang, H. W., Kim, Y. B., & Choi, Y. S. (2020). Extraction of crude gelatin from duck skin: effects of heating methods on gelatin yield. *Poultry science*, *99*(1), 590-596.

[32] Feng, X., Dai, H., Ma, L., Fu, Y., Yu, Y., Zhu, H., ... & Zhang, Y. (2021). Effect of microwave extraction temperature on the chemical structure and oil-water interface properties of fish skin gelatin. *Innovative Food Science & Emerging Technologies*, *74*, 102835.

[33] Duan, R., Zhang, J., Liu, L., Cui, W., & Regenstein, J. M. (2018). The functional properties and application of gelatin derived from the skin of channel catfish (Ictalurus punctatus). *Food chemistry*, *239*, 464-469.

[34] Balti, R., Jridi, M., Sila, A., Souissi, N., Nedjar-Arroume, N., Guillochon, D., & Nasri, M. (2011). Extraction and functional properties of gelatin from the skin of cuttlefish (Sepia officinalis) using smooth hound crude acid protease-aided process. *Food Hydrocolloids*, *25*(5), 943-950.

[35] Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2010). Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. *Food hydrocolloids*, *24*(2-3), 164-171.

[36] Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus). *Food chemistry*, *86*(3), 325-332.

[37] Guo, L., Harnedy, P. A., Zhang, L., Li, B., Zhang, Z., Hou, H., ... & FitzGerald, R. J. (2015). In vitro assessment of the multifunctional bioactive potential of Alaska pollock skin collagen following simulated gastrointestinal digestion. *Journal of the Science of Food and Agriculture*, *95*(7), 1514-1520.

[38] Sántiz-Gómez, M. A., Mazorra-Manzano, M. A., Ramírez-Guerra, H. E., Scheuren-Acevedo, S. M., Navarro-García, G., Pacheco-Aguilar, R., & Ramírez-Suárez, J. C. (2019). Effect of acid treatment on extraction yield and gel strength of whiptail stingray skin gelatin (Dasyatis brevis).*Food Science and Biotechnology*, *28*, 751-757.

[39] Kanwate, B. W., & Kudre, T. G. (2017). Effect of various acids on physicochemical and functional characteristics of gelatin from swim bladder of rohu (Labeo rohita). *Journal of Food Science and Technology*, *54*, 2540-2550.

[40] Usman, M., Sahar, A., Aadil, R. M., & Shahid, M. (2024). Extraction and physicochemical characterization of native and broiler chicken feet gelatin. *Journal of the Science of Food and Agriculture*, *104*(14), 8939-8944.

[41] Shyni, K., Hema, G. S., Ninan, G., Mathew, S., Joshy, C. G., & Lakshmanan, P. T. (2014). Isolation and characterization of gelatin from the skins of skipjack tuna (Katsuwonus pelamis), dog shark (Scoliodon sorrakowah), and rohu (Labeo rohita). *Food hydrocolloids*, *39*, 68-76.

[42] Ab Rahim, H., Ahmad, H., & Ab Rahim, M. H. (2021). Extraction of Gelatin from Different Parts of Gallus Gallus Domesticus. *Current Science and Technology*, *1*(1), 50-55.

[43] Widyasari, R., & Rawdkuen, S. (2014). Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction. *Food and Applied Bioscience Journal*, *2*(1), 85-97.

[44] Rahman, M. N. A., & Jamalulail, S. A. S. K. A. (2012). Extraction, physicochemical characterizations and sensory quality of chicken feet gelatin. *Borneo Science*, *30*, 1-13.

[45] Al-Hassan, A. A. (2020). Gelatin from camel skins: Extraction and characterizations. *Food Hydrocolloids*, *101*, 105457.

[46] da Almeida, P. F., da Silva Lannes, S. C., Calarge, F. A., da Brito Farias, T. M., & Santana, J. C. C. (2012). FTIR characterization of chicken feet gelatin.*Journal of chemistry and chemical engineering*, *6*(11), 1029.

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

مدل‌سازی و بهینه‌یابی فرآیندهای مختلف استخراج ژلاتین پای مرغ با روش سطح پاسخ

هانیه اسمعیلی[۱]، رضا فرهمندفر[۲]، علی معتمدزادگان[۲]، مریم اثنی عشری[۳*]

۱-کارشناسی ارشد، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۲-استاد، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۳-استادیار، بخش تحقیقات فرآوری تولیدات دامی، موسسه تحقیقات علوم دامی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۱۲/۲۸

تاریخ پذیرش: ۱۴۰۴/۰۵/۲۵

کلمات کلیدی:

پای مرغ،
سطح پاسخ،
بازده،
قدرت ژل،
بهینه‌سازی

DOI: 10.48311/fsct.2026.84003.0

* مسئول مکاتبات:

m.asnaashari@yahoo.com

چکیده

مصرف فرآورده‌های جانبی حیوانی در دهه‌های اخیر رشد چشمگیری داشته است. این فرآورده‌ها می‌توانند به محصولات پایداری جهت مصارف کشاورزی و صنعتی تبدیل شوند. یکی از این محصولات پایدار، ژلاتین است. در بین ضایعات کشتارگاهی طیور، پای مرغ منبع خوبی برای تولید ژلاتین می‌باشد ولی مطالعات محدودی در مورد آن وجود دارد. در این تحقیق، به منظور تعیین شرایط بهینه استخراج ژلاتین از پای مرغ به ترتیب اثر چهار متغیر غلظت اسید سولفوریک (۱ تا ۳ نرمال)، نسبت وزن پای مرغ خمیر شده به محلول اسیدی (۱: ۲-۶ وزنی/حجمی)، دمای (۶۰ تا ۹۰ درجه سلسیوس) و زمان استخراج (۱ تا ۵ ساعت) به روش اسیدی و سه متغیر غلظت اسید سولفوریک (۱ تا ۳ نرمال)، توان (۳۶۰ تا ۷۲۰ وات) و زمان مایکروویو (۱۰ تا ۲۰ دقیقه) به روش مایکروویو بر بازده و قدرت ژل مورد بررسی قرار گرفت. نتایج آزمایش‌ها با استفاده از روش طرح مرکب مرکزی سطح پاسخ تجزیه و تحلیل گردید. مدل‌های درجه دوم پیشنهادی برای بازده استخراج و قدرت ژل در روش‌های اسیدی و مایکروویو، R^2 و $Adj\text{-}R^2$ بالا و عدم برازش بی‌معنی داشتند، لذا به خوبی توانستند پاسخ‌ها را پیش‌بینی کنند. شرایط بهینه استخراج اسیدی در حداکثر بازده و قدرت ژل به ترتیب دمای ۷۶/۴۰ درجه سلسیوس، زمان ۳ ساعت، غلظت ۲ نرمال اسید و نسبت وزن پای مرغ به محلول اسیدی ۱:۴ وزنی/حجمی مشخص گردید. نتایج آماری حاصل از آنالیز واریانس خمیرشده فرآیند استخراج به کمک مایکروویو در غلظت ۲ نرمال اسید، توان ۵۴۰ وات و زمان ۲۰ دقیقه بالاترین بازده و قدرت ژل را به دنبال داشت. سپس، نمونه‌های بهینه هر دو روش با ژلاتین تجاری گاوی از نظر میزان الکتروفورز ژل اکریل آمید و رنگ مورد بررسی قرار گرفتند. نتایج حاصل نشان داد که ژلاتین پای مرغ می‌تواند جایگزین مناسبی برای ژلاتین پستانداران باشد.