Optimization of gelatin extraction from chicken deboner residue using RSM method

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Abstract This study aims to investigate the optimization of gelatin extraction from chicken deboner residue. An optimization procedure using a central composite design with three factors (HCl concentration, extraction temperature, and extraction time) was used in order to investigate the effects of these parameters on extraction yield, g-f, viscosity, and lightness. It was found that the optimum conditions for producing gelatin using response surface methodology (RSM) included an acid concentration of 6.73% and an extraction temperature of 86.8 °C for 1.95 h. The predicted responses for these extraction conditions included a yield of 10.2%, a g-f of 526 g, a viscosity of 5.85 cP, and a lightness of 70.0. Verification experiments were conducted under optimal conditions to compare predicted and actual values of the dependent variables. Both actual and predicted values were found to nearly coincide, confirming that the estimation models were capable of reasonably and accurately predicting the dependent variables.

Keywords Optimization · Response surface methodology · Chicken deboner residue · Gelatin

Introduction

Gelatin is an important biopolymer which has been widely used in the food industry (Gilsenan and Ross-Murphy

2000) as an ingredient for increasing the viscosity of aqueous systems; forming aqueous gels; and improving elasticity, consistency, and stability of food products (Sarabia et al. 2000). It can be used in adsorbent pads and for encapsulation, production of hard and soft capsules, wound dressing, and edible film formation, which make it suitable for pharmaceutical and photographic applications (Jongjareonrak et al. 2006). Gelatin is derived from collagen. It is the principal proteinaceous component of the white fibrous connective tissue. Collagen serves as the chief tensile stress bearing elements for animals. This animal protein is isolated in major tissues such as skin tendon and bones, but collagen fibers pervade almost every organ and tissue. Most commercial gelatins are made from the hide of porcines and bovines and to a lesser extent from their bones. Poultry and fish by-products are seldom used as a source of gelatin. Traditional sources of gelatin pose certain problems. For example, Jewish and Muslim communities do not accept pork gelatin (Badii and Nazlin 2006), and beef gelatin is acceptable only if it has been processed according to their religious requirements, which will vary. On the other hand the major defect of fish gelatin is its fishy odor (Cho et al. 2004b). These considerations have encouraged production of gelatin from poultry waste derived by mechanical deboning operations as a replacement for mammalian gelatins.

Mechanically deboning is a unit operation in the poultry processing industry, in which pressure is applied to separate meat from the slurry of ground meat and bones in a mechanical deboner. The separated ground meat is used in the manufacture of comminuted poultry products and the waste material leaving the deboner is a bony residue that has a high content of bone, skin, and connective tissues, depending on the input material. This residue has approximately 20% protein, 30%–40% of which is collagen. The

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gelatinous protein extracts from some turkey deboner residues have been produced and characterized (Fonkwe and Singh 1997).

The aim of this investigation was to optimize gelatin extraction from mechanically deboned chicken residue using response surface methodology (Box and Wilson 1951). RSM is effective in optimizing processing conditions. The basic principle of RSM is to determine model equations that describe the interrelationships between independent and dependent variables (Edwards and Jutan 1997).

Materials and methods

Materials

Mechanically deboned chicken residue from mechanically deboning of broiler chicken was obtained from Zardaneh Meat Products Factory (Isfahan, Iran) and immediately stored as frozen at the plant in 500 g plastic containers at -20°C until use. All chemical and reagents used were of analytical grade.

Proximate composition

Crude protein (Kjeldal), crude fat, moisture, and ash contents of the raw materials were determined in triplicate (AOAC 1990). Crude protein of the samples was expressed as $6.25 \times$ nitrogen content. All values were calculated on a percent wet weight basis (AOAC 1990).

Sample preparation

The 500 g samples were thawed overnight at $8\text{--}10^{\circ}\text{C}$ in a refrigerator. After thawing, they were dried in a ventilation oven at about 39°C for 24 h until moisture was less than 30% and then were defatted using hexane.

Gelatin extraction procedure

The dried and defatted samples were washed with tap water to remove the external impurities. Gelatin extraction was performed as described by Fonkwe and Singh (1997) with slight modifications. Salt and alkali-soluble proteins were extracted from chicken waste using an alkaline solution as follows: 500 g of the sample was placed in a beaker into which 2000 mL of a 1% (w/v) NaCl solution was added. The pH of the slurry was measured by a pH meter (CORNING-EEL, UK) and adjusted with 1 N sodium hydroxide solution to 10.5–10.7. The slurry was continuously mixed for 30 min at room temperature with pH adjustment every 8–10 min. The slurry was filtered through two layers of cheese-cloth to obtain a protein solution and a

wet chicken waste residue. The residue was then soaked in different hydrochloric acid concentrations with a residue/solution ratio of 1:2 (w/v) for 24 h at room temperature (25°C) to let the collagenous material to swell in the residue matrix. The acid treated residue was then separated and rinsed with tap water until a neutral or faintly acidic pH wash water was obtained. Finally, the swollen residue was soaked in distilled water with a residue/water ratio of 1:3 (w/v) and final extraction was carried out in a water bath at different temperatures for different time periods while the sample was being continuous stirred. The resultant mixture was then filtered using multiple layers of cheese-cloth to obtain a gelatinous extract. The extract was demineralized by adding Purolite C-100-E ion-exchange resins and was further filtered in a Buchner funnel with Whatman paper No. 4 (Irwandi et al. 2009). The samples were then air-dried in a convection oven at $40\text{--}42^{\circ}\text{C}$ until moisture was less than 15%.

Measurements of yield and qualitative characterization

Gelatin yield

The extraction yield (YD) was calculated using the following equation:

$$YD(\%) = \frac{\text{dry weight gelatin}}{\text{dry weight chicken waste}} \times 100$$

G-f

G-f was determined according to the AOAC official method 948.21, using rheometry. A 6.67% gel (w/v) was formed by dissolving the dry gelatin in distilled water as its temperature was brought to 62°C in 15 min by placing it in a water bath at 65°C and then cooling the solution in a refrigerator at 10°C (maturation temperature) for 16–18 h (maturation time). G-f, expressed in g value, at 10°C was determined on a texture analyzer (Instron, UK) with a load cell of 5 kN, cross head speed 1 mm/s, equipped with a 12.7 mm diameter flat-faced cylindrical stainless steel plunger. The dimensions of the sample were 3.3 cm in diameter and 6 cm high. G-f was expressed as maximum force (in g) taken when the plunger head penetrated 4 mm into the gelatin gels. The data was reported as the average of three determinations (AOAC 1990).

Viscosity

The viscosities of gelatin solutions (6.67% w/v) were determined according to the Official Procedure of the Gelatin Manufacturers Institute of America using an

Oswald's viscometer (PSL Ltd., Wickford-ESSX, UK) which was held in a water bath to maintain a constant temperature of 60 ± 1 °C.

The time required for 100 mL of solution to pass through the capillary tube of the pipette is the efflux time. The viscosity of each sample at 60 °C with an efflux time t (in sec) was calculated from the following equation:

$$V = \left(At - \frac{B}{t} \right) \times d$$

where:

V	Viscosity, in millipois (mP)
A, B	A and B pipette constants
t	Efflux time, in seconds
solution density =	1.001 (GMIA 1986) for a 6.67% gelatin solution at 60 °C.

Lightness

Gelatin solution (6.67% w/v) was cooled in a refrigerator to 10 °C for 16–18 h. Color was measured by comparison with standards using the HunterLab (Text flash, Data color, USA). The results were expressed as L (lightness) value (Cho et al. 2006).

Experimental design

Processing of gelatin included two important processes, acid treatment and hot-water extraction. In the preliminary studies, 3 factors, namely concentration of HCl (%), X_1 (1.64, 3, 5, 7, and 8.36%), extraction temperature (°C), X_2 (53.18 (53.2), 60, 70, 80, and 86.82 (86.8)°C), and extraction time (hours, X_3) (1.954 (1.95), 4, 7, 10, and 12.046 (12.05) h) were identified as critical variables that had a significant effect on both quantity and quality of the chicken waste gelatin. These were selected as independent variables. The range and center point values of three independent variables were based on the results of preliminary experiments. Gelatin yield (YD, %, Y_1), g-f (GS, g, Y_2), viscosity (V, centipois, Y_3), and lightness (L , Y_4) were selected as dependent variables for a combination of the independent variables as given in Table 1. A central

composite design (CCD, Box and Wilson 1951) was formulated for the optimization of gelatin extraction from chicken. CCD consisted of 2^3 factorial points, six axial points ($\alpha=1.682$), and six replicates of the central point. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

After the experiments had been preformed and the data collected, multiple regression analysis was performed using the Minitab software (14; Minitab Inc., State Collage, Pa., USA) to fit a 2nd-order model to each response. This model can be expressed with uncoded variables (X_1, X_2, X_3):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where, Y is the estimated response for the dependent variables (yield, g-f, viscosity, and lightness); the regression coefficients are B_0 for the constant term; β_i , β_{ii} , β_{ij} are regression coefficients (β_i for the linear terms, B_{ii} for the quadratic terms for a single variable, B_{ij} for the interaction terms, and $i=1$ to 3; and $j=1$ to 3); X_i, X_j are levels of the independent variables; and ε is a random error. The goodness of fit of the polynomial model was evaluated by the coefficient of determination R^2 and R_{adj}^2 . The values of R^2 suggest that the models can explain a high percentage of the variability in the observed data.

Surface plots were developed using the fitted full quadratic polynomial equations obtained by holding one of the independent variables at a constant value (central point) and changing the levels of the other two variables.

Statistical analysis

The Minitab statistical software was used for regression and graphical analyses of the data. After the multifactor analysis of variance and the 2nd- order model prediction determinations, the optimal extraction conditions for obtaining the maximum values of the four responses was obtained by the desirability function approach using the Minitab software. Also, the response surface plots and surface plots were developed by the Minitab software.

Table 1 Independent variables and their coded and actual values used for optimization

Independent variables	Unit	Symbol	Coded levels				
			−1.682	−1	0	+1	+1.682
Acid concentration	%	X_1	1.64	3	5	7	8.36
Extraction temperature	°C	X_2	53.2	60	70	80	86.8
Extraction time	h	X_3	1.95	4	7	10	12.05

Analysis of variance

The statistical significance of the quadratic polynomial model equation was evaluated by the analysis of variance (ANOVA).

Results and discussion

Proximate composition

Crude protein, lipid, moisture and ash content of mechanically deboner chicken residue were $20.985 \pm 0.655\%$, $6.875 \pm 0.115\%$, $55.82 \pm 0.312\%$, $14.315 \pm 0.215\%$, respectively.

Optimization of gelatin processing

Response surface model fitting and diagnostic checking

Response surface methodology or RSM is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the object is to optimize the response (Montgomery and Myers 1995).

As mentioned earlier, gelatin processing has two important steps: acid pretreatment and hot-water extraction. Acid pretreatment removes some of the impurities, fat, and

acid soluble proteins, while hot water extraction causes the hydrolysis of the collagen and its transformation to gelatin. According to our preliminary experiments, acid pretreatment using two times (w/v) the volume of HCl as chicken deboner residue and a pretreatment time of 24 h resulted in consistent yields of gelatin; therefore, the amount of acid solution and time of pretreatment were fixed to two volumes and 24 h, respectively. The concentration of HCl solution was chosen as an independent variable in the first step. In hot-water extraction, using three times (w/v) distilled water as acid pretreated collagen resulted in consistent yields of gelatin. So, the amount of distilled water was fixed to three volumes. The extraction temperature and time were varied as important independent variables. Thus, the independent variables included the concentration of HCl solution as well as hot-water extraction temperature and time.

Experimental results of the 3-factor, 3-level central composite design are shown in Table 2. The response surface regression (RSREG) procedure for Minitab software was employed to fit the quadratic polynomial equation to the experimental data. Using least square regression, Minitab fit a full response surface model for every response investigated, which included all linear, quadratic, and interaction terms.

All the coefficients of the linear (X_1 ; X_2 ; X_3), quadratic (X_1^2 ; X_2^2 ; X_3^2), and interactions (X_1X_2 ; X_1X_3 ; X_2X_3) were calculated for their significance. The estimated regression coefficients of each 2nd-order response surface model in

Table 2 Central composite design and experimental data

Standard order	Acid concentration (X_1)	Extraction temperature (X_2)	Extraction time (X_3)	Yield (Y_1)	Gel strength (Y_2)	Viscosity (Y_3)	Lightness (Y_4)
1	−1	−1	−1	2.33	480	3.17	55.932
2	+1	−1	−1	4.3	560	4.62	58.540
3	−1	+1	−1	6.33	540	5.11	60.646
4	+1	+1	−1	9.34	490	5.23	61.797
5	−1	−1	+1	4.48	550	4.32	58.386
6	+1	−1	+1	6.7	500	5.44	53.442
7	−1	+1	+1	9.21	490	4.06	70.788
8	+1	+1	+1	16.93	450	3.8	60.628
9	−1.682	0	0	5.65	320	4.14	69.012
10	+1.682	0	0	7.53	400	5.69	62.323
11	0	−1.682	0	1.58	440	4.1	55.678
12	0	+1.682	0	16.9	460	2.82	72.028
13	0	0	−1.682	3.87	570	5.8	86.489
14	0	0	+1.682	12.51	490	4.09	55.386
15	0	0	0	7.46	510	4.62	52.820
16	0	0	0	7.22	500	4.42	52.171
17	0	0	0	7.28	500	4.35	53.746
18	0	0	0	7.31	500	4.4	52.795
19	0	0	0	7.17	500	4.49	53.466
20	0	0	0	7.35	510	4.56	52.236

Table 3 Regression coefficients for 2nd order response surface models in terms of uncoded units

Parameter	Term	Coefficient			
		YD	GS	V	L
β_0		0.3365	501.372	4.47234	52.9485
β_1	X_1	1.0538	13.247	0.36881	-1.6145
β_2	X_2	3.9142	3.927	-0.11003	4.0714
β_3	X_3	2.4340	-15.710	-0.24792	0.4045
β_{11}	X_1^2	-0.3002	-34.324	0.16265	4.0263
β_{22}	X_2^2	0.6367	-6.040	-0.35177	3.3848
β_{33}	X_3^2	0.2655	22.245	0.17325	0.5332
β_{12}	X_1X_2	0.4563	-15.000	-0.33875	-0.9024
β_{13}	X_1X_3	0.2588	2.500	-0.08875	-2.4261
β_{23}	X_2X_3	1.1012	5.000	-0.55625	1.3839

terms of uncoded units are presented in Table 3. The linear coefficients (X_1 ; X_2 ; X_3) and the interaction coefficient (X_2X_3) were highly significant ($P<0.01$) but the quadratic coefficient (X_2^2) was significant ($0.01<P<0.05$) for the yield model. The coefficient of quadratic (X_1^2) was highly significant ($P<0.01$) for the gel strength model. The linear coefficient (X_1) and the quadratic coefficient (X_2^2) were highly significant ($P<0.01$) while the coefficient of linear (X_3) and the coefficient of interaction (X_2X_3) were significant ($0.01<P<0.05$) for the viscosity model. The linear coefficients (X_1 , X_2), interaction coefficient (X_1X_3 , X_1X_2) and quadratic coefficient (X_2^2) for the lightness were highly significant ($P<0.01$).

The coefficients of determination (R^2) for Y_1 , Y_2 , Y_3 , and Y_4 were 0.98, 0.69, 0.86, and 0.95 (Table 4), respectively, which indicates that the models are suitable to represent the real relationships among the selected reaction parameters. The values of R^2 for the models of yield and

viscosity were highly significant for the response surface, but extremely high for the lightness model which was significant at $P<0.01$.

Conditions for optimum responses

Three independent variables including HCl concentration (5%), extraction temperature (70 °C), and extraction time (7 h) were chosen as the central condition of the CCD for optimizing the gelatin processing from chicken deboner residue. Uncoded values of independent variables were determined in the preliminary study. To optimize the four dependent variables (yield, g-f, viscosity, and lightness) simultaneously, the desirability function of MINITAB statistical software was used with the responses to be maximized. Table 5 lists the optimization parameters for the multiple responses YD, GS, V, and L. Coded values of the independent variables were concentration of HCl, $X_1=0.865$; extraction temperature, $X_2=1.682$; and extraction time, $X_3=-1.682$. Actual values of the independent variables against coded values were $X_1=6.73$ (%), $X_2=86.8$ (°C), $X_3=1.95$ h, respectively. Predicted values of yield, g-f, viscosity, and lightness were 10.2 (%), 526 (g), 5.85 (cP), and 70.0, respectively.

Cho et al. (2004a) optimized the gelatin processing from shark (*Isurus oxyrinchus*) cartilage by RSM: Predicted maximum yields of 79.9% for gelatin processing were obtained under the conditions of alkali treatment with 1.6 N sodium hydroxide for 3.16 days and hot-water extraction at 65 °C for 3.4 h.

Cho et al. (2004b) also optimized gelatin extraction from dorsal skin of yellowfin tuna (*Thunnus albacares*) fish by RSM. Predicted values of multiple response optimal conditions (alkali treatment with 1.9% sodium hydroxide for 2.9 days and hot-water extraction at 58.1 °C for 4.7 h) were g-f=429.1 and gelatin content=89.7 (%).

Table 4 Analysis of variance (ANOVA) for the 2nd-order response surface model

Source of variation	Df	YD		GS		V		L	
		Sum of squares	P value	Sum of squares	P value	Sum of squares	P value	Sum of squares	P value
Regression	9	325.784	0.000	34943.2	0.081	9.2018	0.003	696.715	0.000
Linear	3	305.305	0.000	5977.5	0.325	2.8624	0.011	264.210	0.000
Square	3	8.576	0.036	26915.7	0.014	2.8831	0.011	363.582	0.000
Interaction	3	11.903	0.014	2050.0	0.724	3.4563	0.006	68.924	0.000
Residual error	10	6.806	–	15236.8	–	1.4996	–	32.427	–
Total	19	332.590	–	50180.0	–	10.7015	–	729.143	–
R^2	–	0.98	–	0.69	–	0.86	–	0.95	–
R_{adj}^2	–	0.96	–	0.42	–	0.73	–	0.91	–
Weight		1		1		1		1	

YD yield, GS gel strength, V viscosity, L lightness

Table 5 Optimization solutions obtained by the response optimizer

Optimal solution			Predicted response				
X ₁ (%)	X ₂ (°C)	X ₃ (h)		YD (%)	GS (g)	V (cP)	L
6.73	86.8	1.95	Predicted value	10.2	526	5.85	70.0
			Individual desirability	0.53	0.84	1.00	1.00
			Composite desirability	0.81			

YD = yield; GS = gel strength;
V = viscosity; L = lightness

Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin was carried out by Kasankala et al. (2007) using response surface methodology (RSM). The predicted responses under the optimum conditions (acid treatment with 1.19% hydrochloric acid for 24 h and water extraction at 52.6 °C for 5.12 h) were 19.8% yield and 267 gg-f.

The response surface plots for multiple responses for dependent variables are shown in Fig. 1.

Response surface plot presents interrelation between two independent variables and one dependent variable while keeping the other independent variable at zero. The effects of three factors on Y_1 (yield) were highly significant ($P<0.01$) with the factor X_2 having the highest effect. G-f was not significantly influenced by these independent variables.

Figure 1. Response surface plots of interactive effects of A, B and C on yield, viscosity and L values (A: acid concentration, B: extraction temperature and C: extraction time).

The effect of acid concentration and extraction time on viscosity was highly significant ($P<0.01$) and significant

($0.01<P<0.05$), respectively. Independent variable X_1 have a stronger influence. The effect of acid concentration and extraction temperature on Y_4 (L) was highly significant ($P<0.01$) with the factor X_2 having the higher effect.

On the other hand, the quadratic coefficient (X_3^2) was not significant, (X_1^2) was highly significant in g-f and lightness models and (X_2^2) was just significant in yield and highly significant in viscosity and lightness models. The interaction coefficients (X_1X_2), (X_1X_3), and (X_2X_3) were highly significant ($P<0.01$) in viscosity, lightness and yield models, respectively, but significant in viscosity model.

Verification of the regression models

Verification experiments were conducted under optimal conditions to compare predicted values and actual values of dependent variables. The actual values repeated three times were yield=10.00, g-f=520.0 (g), viscosity=5.55 (cP), and L=68.21 with 1.00 and 0.95 for the values of the desirability function, respectively. The experimental results are in good agreement with the predicted values, indicating

Fig. 1 Response surface plots of interactive effects of acid concentration, extraction temperature and extraction time on yield, viscosity and L values. **a** Surface plot of yield vs extraction temperature and extraction time (acid concentration: 0), **b** Surface plot of viscosity vs

extraction temperature and extraction time (acid concentration: 0), **c** Surface plot of L vs acid concentration and extraction temperature (extraction time: 0), **d** Surface plot of L vs extraction temperature and extraction time (extraction temperature: 0)

that both actual and predicted values confirm each other and that the models are reasonable and of high accuracy in predicting the values of the dependent variables. In this study, RSM successfully optimized the conditions used in the gelatin extraction and subsequently maximized yield, g-f, viscosity, and lightness. The generated regression models could be used to predict the values of the four investigated dependent variables. Results of this study might serve as a guideline for the larger scale gelatin extraction, as in a pilot plant, to produce gelatin from chicken deboner residue.

Conclusions

Chicken deboner residue gelatin is potentially an additional source of gelatin where religious or ethical reasons prohibit the use of pork products. The manufacture of chicken deboner residue gelatin would also reduce processing wastes and would result in added-value products. The yield of gelatin extraction from chicken deboner residue was not considerable but the extracted gelatin showed high quality in terms of such properties as g-f. The optimum levels of concentration of acid (%), extraction temperature (°C), and extraction time (h) in processing chicken deboner residue gelatin were determined as 6.73 (%), 86.8 (°C) and 1.95 h, respectively, and the predicted values of Y_1 (extraction yield), Y_2 (g-f), Y_3 (viscosity), and Y_4 (lightness) were 10.2 (%), 525.96 (g), 5.85 (cP), and 70.0, respectively.

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