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The effect of processing conditions on physico-chemical properties of whitecheek shark (*Carcharhinus dussumieri*) skin gelatin

Mina Esmaeili Kharyeki[1], Masoud Rezaei[1], Ali Motamedzadegan[2*]

[1]*Department of Fisheries, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Iran*
[2]*Department of Food Science, Sari Agricultural and Natural Resources University, Sari, Mazandaran, P.O. Box 578, Iran*

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Abstract

The optimum conditions for gelatin extraction from whitecheek shark *Carcharhinus dussumieri* skin have been determined by response surface methodology (RSM). The experiment optimization has been conducted by central composite design (CCD), using NaOH concentration (0.01-1 N), HCl concentration (0.01-1 N), and extraction time (3-8 h) as independent variables, and gelatin protein content (%) and viscosity (mPa.s) as dependent variables. The results showed that the NaOH and HCl concentrations had significant effect on crude protein content and viscosity of whitecheek shark skin gelatin ($P < 0.05$). Whereas, extraction time up to 8 h had no significant effect on these two properties ($P > 0.05$). Optimum extraction conditions have been found at 0.92 N NaOH concentration, 0.01 N HCl concentration, and 5.45 h extraction time with 93.1 (%) protein content, and 10.21 (mPa.s) viscosity of the extracted gelatin. The P-value of lack of fit for viscosity was 0.9356 and total regressions P-value was 0.0028 and for protein content were 0.0501 and 0.0346, respectively.

Keywords: Gelatin, Whitecheek shark, Skin, RSM, Protein content, Viscosity

Introduction

Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, and photographic applications because of its unique functional and technological properties (Karim and Bhat 2009). Generally, pig and cow skins and bones are the main sources of gelatin (Kittiphattanabawon et al. 2010). Recent reports indicate that the annual world production of gelatin is nearly 326000 tons, with pig skin-derived gelatin accounting for the highest (46%) production, followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%) (Karim and Bhat 2009). However, with regard to the occurrence of bovine spongiform encephalopathy (BSE) and the fact that Muslims and Jews advocate abstinence from pork and non religious slaughtering, recently the use of fish skin and bone to process gelatin is gaining interest (Ladislaus et al. 2007).

The quality of gelatin depends on its physicochemical properties, which are greatly influenced, not only by the species or tissue from which it is extracted, but also by the severity of the manufacturing method (Johnston-Banks 1990). Compared to mammalian gelatins, fish gelatins have many different properties. The gelling and melting

* Corresponding author. Email: amotgan@yahoo.com. Tel: +98-1512279250.

temperatures of fish gelatins are lower than those for mammals (Gómez-Guillén et al. 2002). Gelatins of many fish species, however, show higher viscosities than it of mammalian gelatins (Liu et al. 2007). Collagen with different compositions from varying species is associated with the temperature of animals living habitat. The melting temperature of gelatin prepared from the skins of warm-blooded animals and warm-water fish species is generally higher than that of gelatin from the skin of fish species living in cold-water, owing to the greater imino acid content and increased proline hydroxylation degree (Jongjareonrak et al. 2006).

Extraction of fish gelatin has been reported for several fish species such as cod (Gudmundsson and Hafsteinsson 1997), hake (Montero et al. 1999), tilapia (Jamilah and Harvinder 2002), Alaska pollock (Zhou and Regenstein 2005), brownstripe red snapper and bigeye snapper (Jongjareonrak et al. 2006), salmon (Arnesen and Gildberg 2007) and rainbow trout (Shahiri Tabarestani et al. 2010). Sharks have been used for shark fin and fillet production in many countries. Skin and cartilage have been generated as by-products. The value-added use of such by-products could pave the way for production of gelatin. This study was designed to optimize the extraction conditions to obtain the highest physicochemical characteristics (crude protein content and viscosity) for gelatin production from whitecheek shark (*C. dussumieri*) skin.

Materials and methods

Shark skin

Whitecheek sharks (*C. dussumieri*) with a total length of 70-100 cm, were transferred from Chabahar, Iran, to Kian Maahi Khazar Co., Ltd. (Babolsar, Iran) at -20 ºC. The sharks were deskined by hand at cold temperature (5 ± 1 ºC) and kept frozen at -30 ºC. The skins were transferred to the lab on ice in less than 1 hr and kept frozen till use.

Gelatin extraction

Gelatin was prepared following the method described by Shahiri Tabarestani et al. (2010) with slight modifications. Frozen skin was thawed overnight at 4 °C and then the residual meat on skin was removed manually. The skin was cut into the 2-3 cm^2 pieces and then washed with cold tap water (8 °C). About 100 g of skin was used for each treatment. To remove non-collagenous proteins, the prepared skin was treated with five volumes (v/w) of cold NaOH (0.01–1 N) at 4 °C. The samples were then washed with extremely tap water until neutral or faintly basic pHs of wash water was obtained. The skins were then soaked in cold HCl (0.01–1 N), at 4 °C with a ratio of 1:5 (w/v). The samples were washed out with cold tap water. Each treatment was repeated three times with a total time of 1 h. For water extraction, five volumes (w/v) of distilled water was added into the sample and then heated at 55 °C (± 0.2) (3-8 h, according to RSM, Table 1) in a water bath (Memmert, WB14, Germany). After the extraction, gelatin solutions were filtered using two layer filter cloth to remove the skin residues. The extracted gelatin was then dried in a freeze-dryer (Operon, FDU-7012, South Korea) for further analysis.

Table 1. Independent variables and their levels for production of whitecheek shark (*C. dussumieri*) skin gelatin

Independent variables	Symbols	Range and levels				
		-1.682	-1	0	+1	+1.682
Concentration of NaOH (N)	X_1	0.01	0.21	0.505	0.8	1
Concentration of HCl (N)	X_2	0.01	0.21	0.505	0.8	1
Extraction time (h)	X_3	3	4.01	5.5	6.99	8

Crude protein content of extracted gelatin

Crude protein content of freeze-dried gelatin was determined by Kjeldahl method (AOAC 2000) with the method number of 928.08.

Viscosity

In order to determine the viscosity, gelatin was dissolved in distilled water (6.67%, w/v) using a water bath at 60 °C for 30 min. Then the viscosity (mPa.s) of 20 ml of gelatin solutions was determined using a Brookfield LVDV-II

viscometer (Brookfield Engineering Laboratories Ltd., Middleboro, MA) with small sample adaptor equipped with a No. 1 spindle at 90 rpm.

Response surface methodology (RSM)

The optimum conditions for gelatin extraction from whitecheek shark skin were determined by response surface methodology (RSM). Optimization was done using central composite design (CCD) as statistical experimental design. CCD in the experimental design consists of 2^3 factorial points, six axial points ($\alpha = \pm 1.682$) and four replicates of the central point (Table 1). Concentration of NaOH (N, X_1), concentration of HCl (N, X_2) and extraction time (h, X_3) have been used as independent variables. The range and center point values of the three independent variables based on the preliminary experiments results are shown in Table 1. The crude protein content (%, Y_1) and viscosity (mPa.s, Y_2) of gelatin were selected as the responses (Table 2).

Analysis of data

The response surface regression (RSREG) procedure of the Statistical Analysis System software (Version 9.1, SAS Institute Inc., USA) was used to fit the following quadratic polynomial equation:

$$Y = ß_0 + \sum_{i=1}^{3} ß_i X_i + \sum_{i=1}^{3} ß_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} ß_{ij} X_i X_j$$

Where Y is the estimated response (crude protein content, and viscosity), $ß_0$ is the constant, $ß_i$; $ß_{ii}$ and $ß_{ij}$ are regression coefficients, and X_i, X_j are levels of the independent variables. After the multi factor analysis of variance and the second order model prediction determinations, the optimal extraction condition was calculated by desirability function of MINITAB statistical software (Version 14; Minitab Inc., State College, PA, USA). The response surface plots were developed using MATLAB software (Version 6.5, The Math Works Inc., Natick, Mass, USA).

Table 2. Central composite design and responses of dependent variables for gelatin extraction from whitecheek shark (*C. dussumieri*) skin to independent variables

Run no.	Coded levels of variable			Response	
	X_1	X_2	X_3	Y_1	Y_2
1	1	1	1	87.0973	2.13
2	1	1	-1	88.5551	1.77
3	1	-1	1	92.3922	6.57
4	1	-1	-1	94.3037	5.71
5	-1	1	1	90.0994	6.88
6	-1	1	-1	87.4741	5.82
7	-1	-1	1	89.0184	7.39
8	-1	-1	-1	85.3292	7.27
9	0	0	1.682	90.0110	3.49
10	0	0	-1.682	91.9696	3.73
11	0	1.682	0	87.3368	4.65
12	0	-1.682	0	91.5407	10.90
13	1.682	0	0	87.7143	2.37
14	-1.682	0	0	90.8373	6.16
15	0	0	0	93.0851	3.25
16	0	0	0	92.9862	6.72
17	0	0	0	92.0728	4.71
18	0	0	0	93.7152	4.80

Y_1 (Crude protein content, %), Y_2 (Viscosity mPa.s), X_1 (Concentration of NaOH, N),
X_2 (Concentration of HCl, N), X_3 (Extraction time, h).

Results and discussion

Optimization of gelatin extraction

Development of response surface model

Experimental results of the central composite design are shown in Table 2. The RSREG procedure for SAS software was used to fit the quadratic polynomial equation to the experimental data. All the coefficients of linear (X_1, X_2, X_3), quadratic (X_1X_1, X_2X_2, X_3X_3), and interaction were calculated for significant differences using *t*-test. The estimated coefficients of all models are presented in Table 3. To develop the fitted response surface model equations (Table 4), all insignificant terms ($P > 0.05$) have been eliminated. The values of R^2 suggest that the quadratic models can explain variabilities in the observed data. Thus, the analysis of variance showed that predicted response surface models were statistically significant ($P < 0.05$).

Analysis of variance

The analysis of variance (ANOVA) was used to evaluate the significance of the quadratic polynomial model equation. Analysis of variance for the quadratic polynomial models is presented in Table 5. The results showed that the linear term for dependent variable of Y_1 and cross product term for dependent variable of Y_2 were not significant, ($P > 0.05$). Whereas, quadratic terms (X_1^2, X_2^2, X_3^2) and total regression models were significant for all dependant variables ($P < 0.05$).

Table 3. Regression coefficients for the response surface models in terms of coded units

Term	Y_1	SE_1	Y_2	SE_2
Intercept	92.986096 ^{S}	0.827	4.859788 ^{S}	0.837
X_1	0.379682 ^{NS}	0.448	-1.285999 ^{S}	0.453
X_2	-1.090533 ^{S}	0.448	-1.527322 ^{S}	0.453
X_3	-0.025300 ^{NS}	0.448	0.146341 ^{NS}	0.435
X_{11}	-1.399508 ^{S}	0.466	-0.169994 ^{NS}	0.472
X_{22}	-1.341782 ^{S}	0.466	1.073629 ^{S}	0.472
X_{33}	-0.792046 ^{NS}	0.466	-0.402066 ^{NS}	0.472
X_{21}	-1.783684 ^{S}	0.586	-0.802500 ^{NS}	0.592
X_{31}	-1.210466 ^{NS}	0.586	0.005000 ^{NS}	0.592
X_{32}	-0.076271 ^{NS}	0.586	0.055000 ^{NS}	0.592

Significant: $P < 0.05$, non-significant: $P > 0.05$.
Y_1 (Crude protein content, %), Y_2 (Viscosity, mPa.s), SE_1 (Standard Error of Y_1), SE_2 (Standard Error of Y_2), X_1 (concentration of NaOH, N), X_2 (Concentration of HCl, N), X_3 (Extraction time, h).

Table 4. Response surface models for processing conditions of gelatin from whitecheek shark (*C. dussumieri*) skin

Response	Quadratic polynomial model	R^2	P-value
Y_1	$Y = 92.986096-1.090533X_2-1.399508X_1^2-1.341782X_2^2 -1.783684X_1X_2$	0.8138	0.0346
Y_2	$Y = 4.859788-1.285999X_1-1.527322X_2+1.073629X_2^2$	0.9074	0.0028

Y_1 (Crude protein content, %), Y_2 (Viscosity, mPa.s), X_1 (Concentration of NaOH, N),
X_2 (Concentration of HCl, N), X_3 (Extraction time, h).

Table 5. Analysis of variance (ANOVA) for the response surface model

Sources		Regression	Linear	Square	Interaction	Lack-of-fit	Pure error	Total error
DF		9	3	3	3	5	3	8
Y_1	SS	96.050530	18.20298	40.626960	37.220589	20.605871	1.373783	21.979654
	PV	0.0346	0.1647	0.0317	0.0392	0.0501	_	_
Y_2	SS	80.674446	54.687283	20.810713	5.176450	2.154954	6.077400	8.232354
	PV	0.0028	0.0007	0.0140	0.2483	0.9356	_	_

Y_1 (Crude protein content, %), Y_2 (Viscosity, mPa.s), X_1 (Concentration of NaOH, N), X_2 (Concentration of HCl, N), X_3 (Extraction time, h), DF (Degree of Freedom), SS (Sum of Square), PV (P-Value).

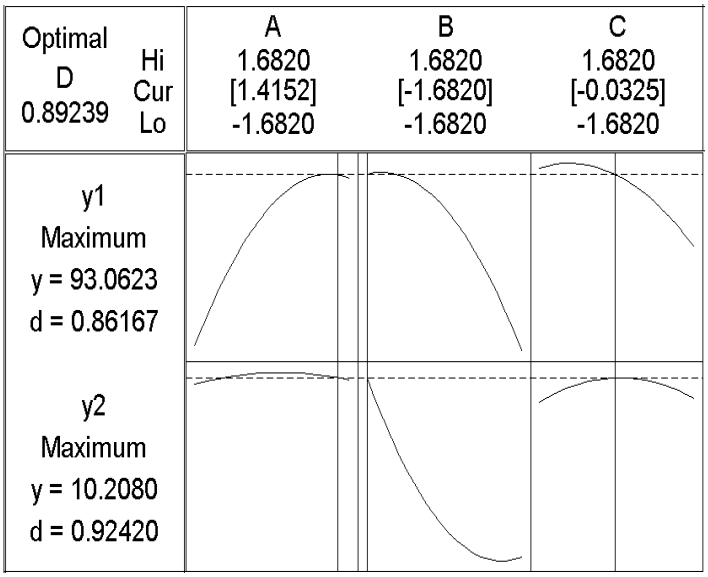

Fig. 1. Predicted values of multiple response optimal conditions

Multiple response optimization

According to preliminary study, three factors including concentration of NaOH (X_1), concentration of HCl (X_2), and extraction time (X_3), were identified as important variables that had significant effects on different characteristics of gelatin extracted from whitecheek shark skin. To optimize two dependant variables (Y_1 and Y_2) simultaneously, desirability function of MINITAB statistical software were defined as following conditions; goal (maximize), target (Y_1 = 94.303 and Y_2 = 10.90). Coded values of the independent variables were concentration of NaOH, X1 = 1.4152; concentration of HCl, X_2 = -1.682 and extraction time, X_3 = -0.0325 (Fig. 1). Actual values of independent variables against coded values were X_1 = 0.92 (N), X_2 = 0.01 (N), X_3 = 5.45 (h), respectively. The predicted values of multiple response optimal conditions were Y_1 = 93.062, Y_2 = 10.208 with 0.89 of the value of desirability function.

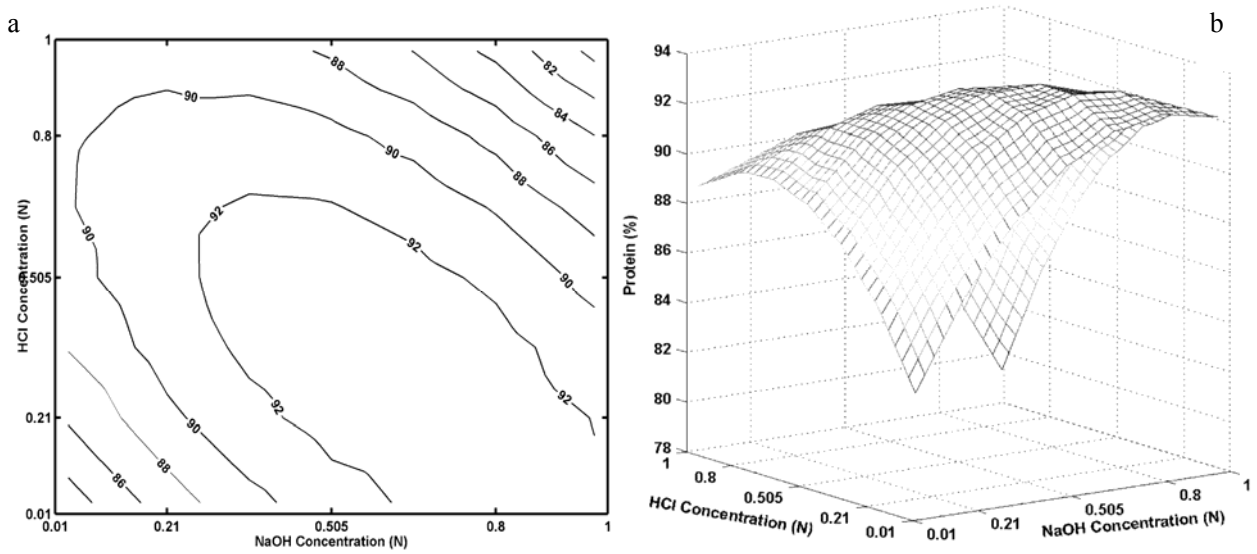

Fig. 2. The response surface plot (a and b) of crude protein content of whitecheek shark skin gelatin

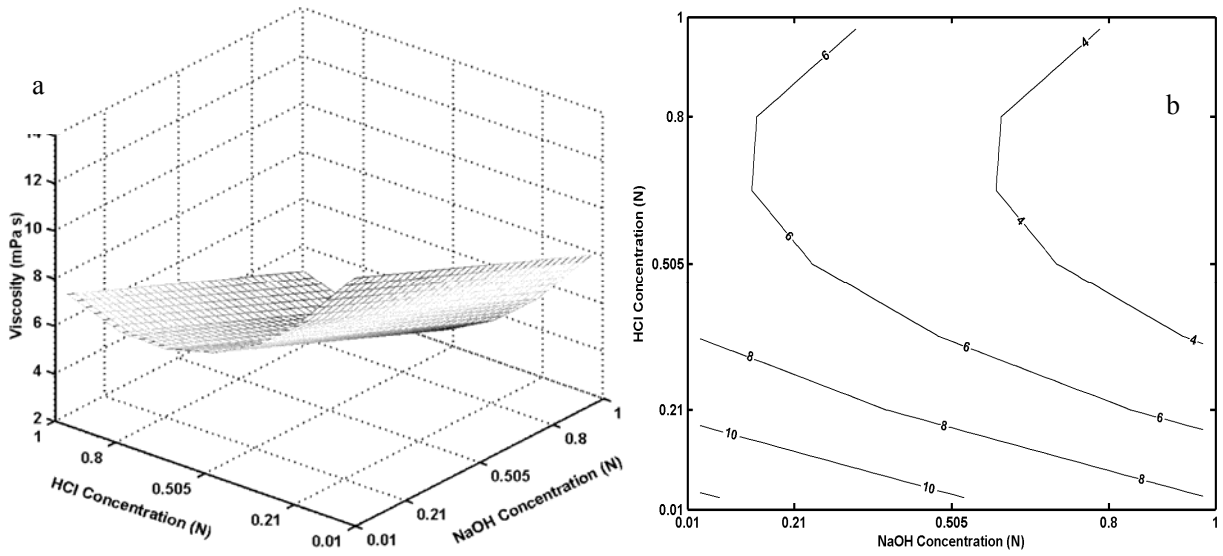

Fig. 3. The response surface plot (a and b) of viscosity of whitecheek shark skin gelatin

Effect of extraction conditions on gelatin properties

Crude protein content of gelatin

The essential constituent of gelatin is protein. The protein content of commercial gelatin is ranged between 85 and 92%, the remainder being mineral salts and any moisture still left after drying (Schrieber and Gareis 2007). The crude protein content of gelatin powder in this study was ranged between 85.32-94.30%, that is similar to protein content of commercial gelatins. The multiple regression model that developed to predict protein content of whitecheek shark skin gelatin, indicates the adequacy of the selected model to explain the variations in crude protein content ($R^2 = 0.8138$). The significant variables affecting protein content were the linear and quadratic terms of HCl concentration and quadratic term of NaOH concentration (Table 4). Significant interactions were found between concentrations of NaOH and HCl ($P < 0.05$). The independent variable of time had no significant effect on protein content of extracted gelatin ($P > 0.05$). The response surface plot (Fig. 2) shows that protein content of extracted

gelatin has increased by increasing in acid and alkali concentration from 0.01 to 0.8 N and decreased in concentration of higher than 0.8 N.

Viscosity

Viscosity is one of the most important rheological properties of gelatin solutions. The significant variables affecting viscosity were the concentration of HCl, and NaOH. Extraction time was not significant as showing in Table 4. Figure 3 shows that the viscosity of gelatin decreases by increasing in acid and alkali concentrations. This result is in agreement with that reported by Boran and Regenstein (2009) for silver carp skin. the viscosity of commercial gelatins have been reported to be from 2 to 7 mPa s for most cases and up to 13 mPa s for specialized ones (Johnston-Banks 1990). In this study viscosity of whitecheek shark skin gelatin samples ranged among 1.77-10.90 mPa.s, depending on the extraction conditions (Table 2), which is similar to commercial gelatins.

Conclusion

The results obtained by this study sugges that whitecheek shark skin might successfully be used in gelatin production as an alternative raw material in place of pork skin where high viscosity gelatin is needed. The concentration of NaOH and HCl had significant effect on crude protein content and viscosity. Optimum extraction conditions were found to be 0.92 (N) NaOH concentration, 0.01 (N) HCl concentration and 5.45 (h) extraction time, with 93.062 (%) crude protein content and 10.208 (mPa.s) viscosity of the extracted gelatin.

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