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Processing Optimization of Gelatin from Rockfish Skin Based on Yield

Hyung Jun Kim, Min Seok Yoon, Kwon Hyun Park, Joon Ho Shin, Min Soo Heu[1] and Jin-Soo Kim*

Department of Seafood Science and Technology/Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Korea
[1]Department of Food and Nutrition/Institute of Marine Industry, Gyeongsang National University, Jinju 660-701, Korea

The study was performed to optimize the processing conditions (alkali concentration, extraction time, and temperature) for rockfish skin gelatin based on yield using response surface methodology and comparison of the physicochemical properties with those of rockfish skin gelatin pretreated and extracted under ordinary conditions (alkali treatment concentration: 1.0 M; extraction time: 2 hr; extraction temperature: 60°C). Predicted maximum gelatin yield of 19.1% and gelatin content of 87.8% were obtained by extraction at 106.6°C for 69.0 min after pretreatment with 1.1 M calcium hydroxide. Yield of gelatin extracted under high temperature/high pressure (G-HT/HP) was 54% higher than that extracted under ordinary temperature/time (G-OT/T). However, G-HT/HP was inferior in gel strength and gelling point to (G-OT/T), but comparable in transmission. Based on the physicochemical properties, G-HT/HP was unsuitable for use in products requiring higher physical properties, but could be useful for health-functional foods.

Key words: Rockfish skin gelatin, Gelatin, Rockfish, Fish skin gelatin, Seafood by-products

Introduction

Gelatin can be modified into biologically active peptides by protease treatment, and the resultant peptides generally exhibit activities as potential inhibitors of angiotensin I-converting enzyme and antioxidants against lipid peroxidation (Kim et al., 2001; Mendis et al., 2005). These characteristics suggest that use of gelatin can be extended even further to health-functional foods that are not reliant on its physical properties, as well as various ordinary foods, pharmaceutical applications, and other industrial applications requiring higher physical properties (Kim and Park, 2004). Most commercial gelatin (95%) is made from porcine and bovine hides (Cho et al., 2005). Health-conscious consumers, however, are reluctant to use collagen extracted from land animals due to the recent outbreaks of bovine spongiform encephalopathy (BSE), foot-and-mouth disease, and avian flu. Therefore, raw materials from fishery products have attracted attention as sources of consumer-friendly gelatin (Fernanddez-Diaz et al., 2003).

For application in various industrial products such as ordinary foods, cosmetics, and pharmaceutics, fish gelatin must have rheological properties similar to gelatin from land animals. However, fish gelatin does not readily show similar rheological properties to that from land animals. Therefore, for fish gelatin to be used as a material in ordinary foods, cosmetics, pharmaceutical products, and other products requiring higher rheological properties, it should be modified by chemical and enzymatic methods, and this may be associated with problems regarding safety or cost. One efficient way to utilize fish gelatin is as a resource for health-functional foods without requirements for specific rheological properties (Kim et al., 1997).

Molecular structure of collagen consists of three polypeptides, chains, wound together in a tight triple helix (Kim, 1992), which is loosened by heating and pressurization. Therefore, higher extraction temperature and longer extraction time increase yield and

*Corresponding author: jinsukim@gnu.ac.kr

reduce contamination of the resultant gelatin, but adversely affect the rheological properties. Therefore, the processing conditions should be optimized for economical production of gelatin as a resource for functional ingredients.

Response surface methodology (RSM) is a set of mathematical and statistical techniques widely used to determine the effects of multiple variables and to optimize different processes (Yang et al., 2007). RSM is a mathematical modeling technique that relates independent and dependent variables and establishes regression equations describing the inter-relationships between input parameters and output properties (Cho et al., 2004; Yang et al., 2007). Therefore, RSM is often applied to optimize the conditions for extracting gelatin from various sources (Cho et al., 2004; Cho et al., 2005; Yang et al., 2007).

In Korea, the annual rockfish harvest exceeds 39,500 M/T, which is mainly consumed as sliced raw fish. However, over 65% of rockfish harvest is processing waste, which includes, e.g., bone, skin, fins, internal organs, and head. Rockfish skin contains considerable amounts of collagen, which can be used as a potential resource for collagen and gelatin extraction, but the efficient use of rockfish skin as a human food has not been widely studied. Most of the rockfish skin is conventionally used to produce fish meal and fertilizer or is directly discharged into estuaries, resulting in environmental pollution. New approaches must be used to upgrade the processing of waste to food-grade ingredients, such as collagen and gelatin (Kim and Park, 2005). Rockfish skin has attracted attention as a potential consumer-friendly collagen and gelatin resource.

Although efforts are being made to utilize the skin of various marine animals, such as yellowfin tuna skin (Cho et al., 2005), yellowfin sole skin (Kim, 1992), dover sole skin (Kim, 1992), harp seal skin (Arnesen and Gildberg, 2002), megrim skin (Gomez-Guillen and Montero, 2001), Atlantic salmon skin (Arnesen and Gildberg, 2007), cod skin (Gudmundsson and Hafsteinsson, 1997), shark skin (Hamada, 1990), black tilapia skin (Jamilah and Harvinder, 2002), red tilapia skin (Jamilah and Harvinder, 2002), filefish skin (Kim and Cho, 1996), Alaska pollock skin (Regenstein et al., 2003), Nile perch skin (Muyonga et al., 2004), and conger eel skin (Kim et al., 1996), as well as fish bone, such as Nile perch bone (Muyonga et al., 2004), as gelatin resources, no research has been conducted with regard to utilization of rockfish skin.

The objective of this study is optimize the processing conditions (alkali concentration, extraction temperature, and time) of rockfish skin gelatin to achieve high yields using RSM and to compare the characteristics of the gelatin prepared at high temperature with those of gelatin extracted under ordinary conditions (alkali concentration: 1.0 M; extraction temperature: 60°C; extraction time: 2 hr).

Materials and Methods

Materials

Rockfish (*Sebastes hubbsi*) skin, a by-product of sliced raw fish, was obtained from a fisheries manufactory of Geoje National Federation Fisheries Cooperatives (Geoje, Korea) in December 2007. The skin was mechanically separated, and adhering muscle was removed by hand. After thoroughly washing with running tap water, the skin was placed in polyethylene bags and stored at -25°C until used.

All reagents used in this study were of analytical grade and used without further purification.

Extraction and preparation of gelatin

Rockfish skin was washed with cold tap water for 10 min and sieved to remove all soluble proteins and foreign materials. The washing process was repeated three times. The washed rockfish skin was treated with 10 volumes (v/w) of alkaline solution (0.33-1.67 M calcium hydroxide, $Ca(OH)_2$) with stirring (No. 1; Lab Tech., Daejeon, Korea) at 5°C for 2 hr to remove non-collagenous materials and to inactivate enzymes, and then washed with cold tap water to remove $Ca(OH)_2$. After washing, the skin was neutralized with 1.0 M acetic acid and rewashed with cold tap water. Gelatins were extracted at 83.2 -117.0°C for 9.5-110.5 min in an autoclave (MAC-6100; EYELA, Tokyo, Japan) to obtain a high yield and at 60°C for 120 min in a water bath (BS-21; Jeio Tech, Daejeon, Korea) for comparison with the resultant gelatin under optimal conditions after adding three volumes (v/w) of distilled water to the pretreated skin. Both extracted gelatin solutions were put into activated carbon for discoloration and deodorizing, and centrifuged at 5,300×*g* at 20°C for 15 min. The upper layers were vacuum-filtered with filter paper (Whatman no. 3; Whatman, Maidstone, Kent, UK) and then dried at 50°C for 14-20 hr.

Experimental design

Central composite design (CCD) (Box and Wilson, 1951) was adopted for optimization of gelatin extraction from rockfish skin. CCD consisted of 17 samples, such as factorial points; 8, star points; 6 and central points; 3 (Table 4). Processing of gelatin

consisted of alkali treatment and hot-water extraction (Cho et al., 2005). Concentration (M, X_1) of alkali ($Ca(OH)_2$) to remove foreign materials and to inactivate enzymes, temperature (°C, X_2), and time (min, X_3) in hot-water extraction were chosen as independent variables. The ranges and center point values of three independent variables were based on the results of preliminary experiments (Table 1). Gelatin content (%, Y_1) and yield (%, Y_2) were selected as dependent variables (Table 2). Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

Gelatin content and yield

Gelatin content was estimated by measuring hydroxyproline content according to the method of Sato et al. (1991) using a conversion factor of 12.82. Hydroxyproline content was determined by the method described in ISO 3496-1978 (1978), with slight modifications. Dried gelatin (10 mg) was placed in test tubes, and 3 mL of 6 N HCl was added. The sample solutions were hydrolyzed at 110°C for 12 hr using a dry bath (HF 21; Yamato Co., Tokyo, Japan). After acid hydrolysis, the sample solutions were neutralized with 6 N NaOH, filtered with a microfilter, and mixed with 2 mL of acetate/citrate buffer (pH 6.0) before reaching a volume of 25 mL with 0.3 M NaCl. Oxidant solution was prepared by mixing one volume of 7% (w/v) chloramine T (sodium salt of *p*-toluene sulfonchloramide) with four volumes of acetate/citrate buffer (pH 6.0). Aliquots were transferred into test tubes, and then isopropanol (300 L) and oxidant solution (600 L) were added and kept at room temperature for 4 min. Ehrlich's reagent solution (4 mL) was then added to each tube, mixed, and heated at 60°C for 25 min in a water bath (BS-21; Jeio Tech). Absorbance of the solution was measured with a spectrophotometer (UV-140-02; Shimadzu Co., Kyoto, Japan) at 660 nm. The hydroxyproline content of the sample solution was calculated from a standard calibration curve using analytical-grade hydroxylproline purchased from Sigma-Aldrich Co. (St. Louis, MO).

Yield of gelatin was calculated as (the resultant gelatin weight/weight of pretreated rockfish skin

Table 1. Experimental range and values of the independent variables in the central composite design for gelatin extraction from rockfish skin

Independent variable	Symbol	Range and levels				
		-1.682	-1	0	+1	+1.682
Concentration of $Ca(OH)_2$ (M)	X_1	0.33	0.60	1.00	1.40	1.67
Extraction temperature (°C)	X_2	83.2	90.0	100.0	110.0	117.0
Extraction time (min)	X_3	9.5	30.0	60.0	90.0	110.5

Table 2. Central composite design and responses of dependent variables for gelatin extraction from rockfish skin to independent variables

Run no.	Coded levels of variable			Response		
	X_1 $Ca(OH)_2$ (M)	X_2 Temp. (°C)	X_3 Time (min)	Y_1	Y_2	Coefficients assessed by
1	-1	-1	-1	87.1	13.1	Fractional factorial design (8 points)
2	1	-1	-1	90.2	14.3	
3	-1	1	1	85.5	17.8	
4	1	1	1	87.1	18.4	
5	-1	-1	-1	85.6	13.7	
6	1	-1	-1	89.4	14.5	
7	-1	1	1	82.2	18.6	
8	1	1	1	87.5	19.6	
9	-1.682	0	0	82.6	14.4	Star points (6 points)
10	1.682	0	0	88.6	17.0	
11	0	-1.682	-1.682	89.8	14.3	
12	0	1.682	1.682	87.0	19.3	
13	0	0	0	89.0	15.8	
14	0	0	0	87.0	17.9	
15	0	0	0	88.1	17.8	Central points (3 points)
16	0	0	0	88.3	17.7	
17	0	0	0	88.2	17.6	

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %).

used)×100.

Proximate composition and heavy metal contents

The moisture content was determined by oven-drying at 105°C, total protein was determined by the semimicro-Kjeldahl procedure, and crude ash was determined by incineration in a muffle furnace at 550°C in accordance with the Association of Official Analytical Chemists methods (AOAC, 1990). Total lipid was extracted into a methanol–chloroform mixture according to the method of Bligh and Dyer (1959). The mercury content was determined by the combustion gold amalgamation method (KFDA, 2006) using a mercury analyzer (SP-3A; Nippon Instrument Co., Tokyo, Japan). Other heavy metals, such as Pb, Cd, and Cr, were determined by the wet ash method (Tsutagawa et al., 1994) using an inductively coupled plasma (ICP) spectrophotometer (Atomscan 25; Thermo Electron Co., Waltham, MA).

Molecular weight profile

Twenty-microliter aliquots of gelatin extracted under high temperature/high pressure (G-HT/HP) or under ordinary temperature/time (G-T/T) were injected onto a Shodex protein KW-804 column (i.d. 8 mm×300 mm; Showa Denko, Tokyo, Japan) equilibrated with 50 mM sodium phosphate buffer (pH 6.0) containing 100 mM NaCl and analyzed using a HPLC (LC-10ATvp; Shimadzu) at a flow rate of 1 mL/min. The elution profile of protein was monitored using a UV-Vis detector (SPD-10AVvp; Shimadzu) at 280 nm. The molecular weights of standard proteins (Sigma-Aldrich Co.) used were as follows: aprotinin (6,500 Da), cytochrome *c* (12,400 Da), carbonic anhydrase (29,000 Da), bovine serum albumin (66,000 Da), and alcohol dehydrogenase (150,000 Da).

Gel strength

Gel strength was determined as described by Zhou and Regenstein (2004) with slight modifications. Gelatin was dissolved in distilled water (6.7%, w/v) at 60°C for 30 min until completely dispersed and poured up to a height of 2.6 cm into a beaker (20 mL in capacity, 3.0 cm in diameter, and 4.0 cm in height). The beaker was covered with Parafilm (Pechiney Plastic Packaging Co., Chicago, IL) to prevent drying of gelatin gel and matured at 5°C for 16-18 hr. The strength of the resultant gel, expressed in grams, was measured at 5°C using a rheometer (CR-100D; Sun Scientific Co., Tokyo, Japan) with depression of 4 mm at a rate of 20 mm/min with a probe 10 mm in diameter and a load cell of 10 kg.

Transmission and Hunter color value

Transmission of gelatin sol was measured using a UV-visible recording spectrophotometer (Model UV-140-02; Shimadzu) according to the method of the Pharmaceutical Society of Japan (1980).

The Hunter color value of gelatin powder was measured as L, a, and b using a colorimeter (Model ZE-2000; Nippon Denshoku Industries Co., Tokyo, Japan); the L, a, and b values of a standard white plate were 91.6, 0.28, and 2.69, respectively.

Gelling point

Gelling point was determined based on the viscosity according to the method of Kolodziejska et al. (2008) with slight modifications. The viscosity was measured continuously during cooling of gelatin solution from 40°C to 5°C at a rate of 1°C/min. The temperature at which a sharp increase in viscosity appeared was assumed to be the gelling point. The viscosity of gelatin solutions was determined using 10 mL of 6.7% gelatin solution with a Brookfield viscometer (LVDVII+; Brookfield Engineering Laboratories, Middleboro, MA) with spindle number 7 at 140 rpm.

Data analysis

Response surface regression (RSREG) procedure implemented with SAS version 9.1 (SAS Institute Inc., Cary, NC) was used to fit the following quadratic polynomial equation:

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i X_i+\sum_{i=1}^{3}\beta_{ii}X^2{}_i+\sum_{i=1}^{2}\sum_{j=i+1}^{1}\beta_{ij}X_iX_j$$

where Y is the dependent variable (gelatin content and yield); β_0 is a constant; β_i, β_{ii}, and β_{ii} are regression coefficients; and X_i and X_j are the levels of the independent variables. The Ridge Max option of the RSREG procedure was used to compute the estimated ridge of the optimum response for increasing radii from the center of the original design. Multiple response optimizations was heuristically calculated using the desirability function of MINITAB statistical software ver. 14 (Minitab Inc., State College, PA) to determine the conditions simultaneously satisfying two dependent variables (Y_1 and Y_2). Response surface plots were developed using Maple software ver. 7 (Maple Inc., Waterloo, ON, Canada) and represented a function of two independent variables while keeping the other independent variables at the optimal values.

Results and Discussion

Diagnostic check of the fitted model

RSREG procedure with SAS software was used to fit the quadratic polynomial equation using the experimental data (Table 2). All coefficients of linear (X_1, X_2, and X_3), quadratic (X_1X_1, X_2X_2, and X_3X_3), and interaction terms (X_1X_2, X_1X_3, and X_2X_3) were calculated for significance with *t*-statistics, and the results are shown in Table 3. In Y_1 (gelatin content, %), all the coefficients of the linear terms were significant ($P<0.05$) in models, while those of quadratic and interaction terms except for X_1X_1 ($P=0.001$) and X_1X_3 ($P=0.033$) were not significant ($P>0.05$). In Y_2 (yield of gelatin), all the linear coefficients were significant ($P<0.05$) in models, while those of quadratic and interaction except for X_1X_1 ($P=0.008$) were not significant.

Fitted response surface models were made by eliminating all insignificant terms ($P>0.05$), the results are shown in Table 4. In the fitted response surface models of Y_1, X_2 (extraction temperature, °C), X_3 (extraction time, min) and X_1X_1, the terms had negative effects, while X_1 (concentration of $Ca(OH)_2$, M) and X_1X_3 terms had positive effects. In the fitted response surface models of Y_2, the X_1, X_2, and X_3 terms had positive effects, while the X_1X_1 term had a negative effect. The coefficients of multiple determinations (R^2) for Y_1 and Y_2 were 0.970 and 0.955, respectively. The values of R^2 for the models were very high for the response surface and significant at $P<0.05$. The results of R^2 suggested that the models were suitable for representing the real relationships among the selected reaction parameters (Cho et al., 2005).

Analysis of variance (ANOVA)

Statistical significance of the quadratic polynomial model equation was investigated by ANOVA and the results are shown in Table 5. All the dependent variables, Y_1 (gelatin content, %) and Y_2 (yield of gelatin, %), were significant in the linear ($P=0.000$ for Y_1 and Y_2) and quadratic ($P=0.044$ for Y_1 and $P=0.004$ for Y_2) terms, while they were not significant in the interaction terms ($P=0.930$ for Y_1 and $P=0.154$ for Y_2). The lack of fit, which indicates the fitness of the model, in all the dependent variables was also significant at a 95% probability level. The results suggested that quadratic polynomial models were suitable for fitting extraction conditions of gelatin from rockfish skin. The canonical form of the quadratic polynomial model was investigated to determine the type of stationary point (Table 6). The stationary point of the quadratic polynomial model is divided into three types by the eigenvalue sign: maxima point (all negatives in the eigenvalue of the canonical form), minimum point (all positives in the

Table 3. Estimated coefficients of the fitted quadratic polynomial equation for different response based on t-statistic

	Y_1		Y_2	
	Coefficient	*P*-value	Coefficient	*P*-value
Intercept	88.2294	0.000	17.7080	0.000
X_1	1.7494	0.000	0.5838	0.015
X_2	-1.0770	0.000	1.9923	0.000
X_3	-0.6271	0.006	0.4636	0.039
X_1X_1	-1.0204	0.001	-0.7347	0.008
X_1X_2	-0.0000	1.000	-0.0500	0.841
X_1X_3	0.5500	0.033	-0.0000	1.000
X_2X_2	-0.0305	0.866	-0.3458	0.130
X_2X_3	-0.0750	0.728	0.1500	0.551
X_3X_3	-0.1719	0.358	-0.3281	0.148

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %), X_1 (concentration of $Ca(OH)_2$, M), X_2 (extraction temperature, °C), X_3 (extraction time, min).

Table 4. Response surface model for extraction conditions of gelatin from rockfish skin

Response	Quadratic polynomial model	R^2	*P*-value
Y_1	$88.2294 + 1.7494X_1 - 0.0770X_2 - 0.6271X_3 - 1.0204X_1^2 + 0.5500X_1X_3$	0.970	0.0150
Y_2	$17.7080 + 0.5838X_1 + 1.9923X_2 + 0.4636X_3 - 0.7347X_1^2$	0.955	0.0206

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %), X_1 (concentration of $Ca(OH)_2$, M), X_2 (extraction temperature, °C), X_3 (extraction time, min).

Table 5. Analysis of variance (ANOVA) for response of dependent variables (Y_1 and Y_2)

Responses	Sources	DF	SS	MS	F-value	*P*-value
Y_1	Model	9	68.38	7.60	16.54	0.001
	Linear	3	61.80	20.60	44.85	0.000
	Quadratic	3	6.38	2.13	4.63	0.044
	Cross-product	3	0.20	0.07	0.15	0.930
	Residual	7	3.22	0.46	-	-
	Lack of fit	5	3.20	0.64	63.90	0.015
	Pure error	2	0.02	0.01	-	-
	Total	16	71.60	8.06	-	-
Y_2	Model	9	78.18	8.69	25.27	0.000
	Linear	3	63.01	21.00	61.09	0.000
	Quadratic	3	12.71	4.24	12.32	0.004
	Cross-product	3	2.47	0.82	2.3	0.154
	Residual	7	2.41	0.34	-	-
	Lack of fit	5	2.39	0.48	47.73	0.021
	Pure error	2	0.02	0.01	-	-
	Total	16	80.59	9.03	-	-

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %).

Table 6. Canoncial form for extraction conditions of gelatin from rockfish skin

Y_1	$Y_1 = 88.2400 - 0.0461(\omega_1)^2 - 0.3050(\omega_2)^2 - 3.1153(\omega_3)^2$
Y_2	$Y_2 = 17.6840 - 0.7255(\omega_1)^2 - 1.1516(\omega_2)^2 - 2.0826(\omega_3)^2$

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %).

Table 7. Optimal conditions for gelatin extraction from rockfish skin

Dependent variables	Quadratic polynomial model	Critical value		Predicted value	Stationary point
		Coded	Uncoded		
Y_1	X_1	-1.682	1.67 M	87.8%	Maximum
	X_2	-1.682	83.2°C		
	X_3	-1.682	110.5 min		
Y_2	X_1	-0.170	1.07 M	19.1%	Maximum
	X_2	-1.682	117.0°C		
	X_3	-0.864	85.9 min		
Multiple response optimization	X_1	-0.284	1.11 M	-	-
	X_2	-0.663	68.8 min		
	X_3	-0.293	106.6°C		

Y_1 (gelatin content, %), Y_2 (yield of gelatin, %), X_1 (concentration of $Ca(OH)_2$, M), X_2 (extraction temperature, °C), X_3 (extraction time, min).

eigenvalue of the canonical form), and saddle point (both negative and positive eigenvalues of the canonical form). All the stationary points of the two quadratic polynomial models (Y_1 and Y_2) fitted in the study were maxima because all the eigenvalues of Y_1 and Y_2 were negative.

Conditions for optimum response

Three independent variables, the concentration of $Ca(OH)_2$ (1.00 M), extraction temperature (100°C), and extraction time (60 min), were adopted as the central conditions of the central composite design (CCD) for optimizing gelatin extraction from rockfish skin. Optimal conditions of coded and uncoded values of multiple responses are shown in Table 7. The maxima independent variables regarding each Y_1 (gelatin content, %) and Y_2 (yield of gelatin, %) were 1.682 and 0.170, respectively; for X_1; -1.682 and 1.682, respectively, for X_2; 1.682 and 0.864 for X_3 in coded values; and 1.67 M and 1.07 M, respectively, for X_1; 83.2°C and 117.0°C, respectively, for X_2; and 110.5 min and 85.9 min for X_3 in uncoded values. Differences were found in the critical values of the dependent variables. These results probably were

attributable to extraction at high temperature/high pressure for a long time, which led to superabundant extraction of non-collagenous materials, such as elastin, as well as gelatin from rockfish skin (Kim, 1992). To simultaneously optimize two dependent variables, the desirability function in the MINITAB statistical software was defined as follows: goal (maximize), target (Y_1=90.2% and Y_2=19.6%). The optimal independent variables regarding the dependent variables (Y_1 and Y_2) were 0.284 for X_1, 0.663 for X_2, and 0.293 for X_3 in coded values and 1.1 M for X_1, 106.6°C for X_2, and 68.8 min for X_3 in uncoded values. Predicted values of Y_1 and Y_2 of gelatin prepared under optimal conditions as above were 87.8% and 19.1%, respectively.

Response surface plot and influence factor

Estimated response function and the effects of independent variables (X_1, X_2, and X_3) on Y_1 and Y_2 are shown in Fig. 1 and Fig. 2, respectively. Gelatin processing was divided into two important processes: alkali treatment to remove non-collagenous materials and inactivate enzymes, and heating to extract gelatin (Cho et al., 2005). X_1 (concentration of $Ca(OH)_2$) is the most important factor in the various alkali treatment conditions and X_2 (extraction temperature) and X_3 (extraction time) are also important factors in the various extraction conditions in an autoclave. The response surface plot presents the interrelationship between two independent variables while retaining the other one independent variable at the optimal value. As the coded values of all independent variables except for partial coded values (0 to +1.682) of X_1 closed from -1.682 to 1.682, the gelatin content increased and the rate of increase was faster in X_2 than in X_3. However, as the coded values of X_1 ranged from 0 to +1.682, no difference in the gelatin content was found. As the coded values of all independent variables except for partial coded values (0 to +1.682) of X_1 and X_3 closed from -1.682 to +1.682, the yield increased. However, as the coded values of X_1 and X_3 closed from 0 to +1.682, the yield of gelatin decreased in X_1, while no difference occurred in X_3. The yield decreased with closing from 0 to +1.682 in the coded values of X_1, which probably occurred because a high concentration of alkali caused protein loss (Cho et al., 2005). Considering the three response plots, all independent

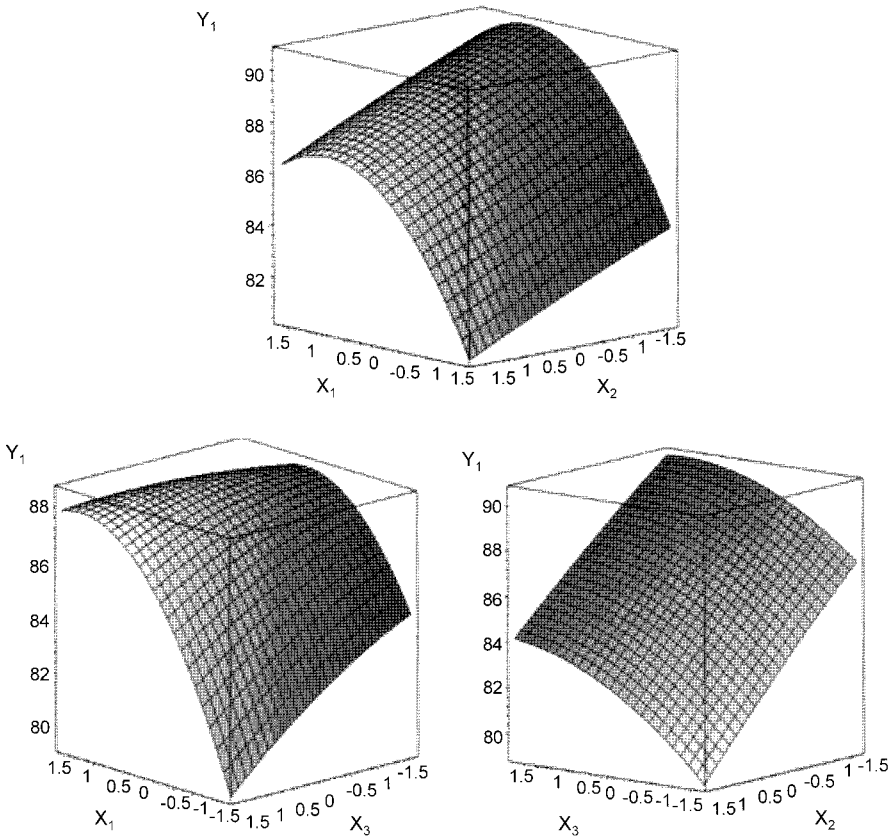

Fig. 1. Response surface plots for optimization of gelatin extraction from rockfish skin based on Y_1 (gelatin content, %). X_1 (concentration of $Ca(OH)_2$. M), X_2 (extraction temperature, °C), X_3 (extraction time, min).

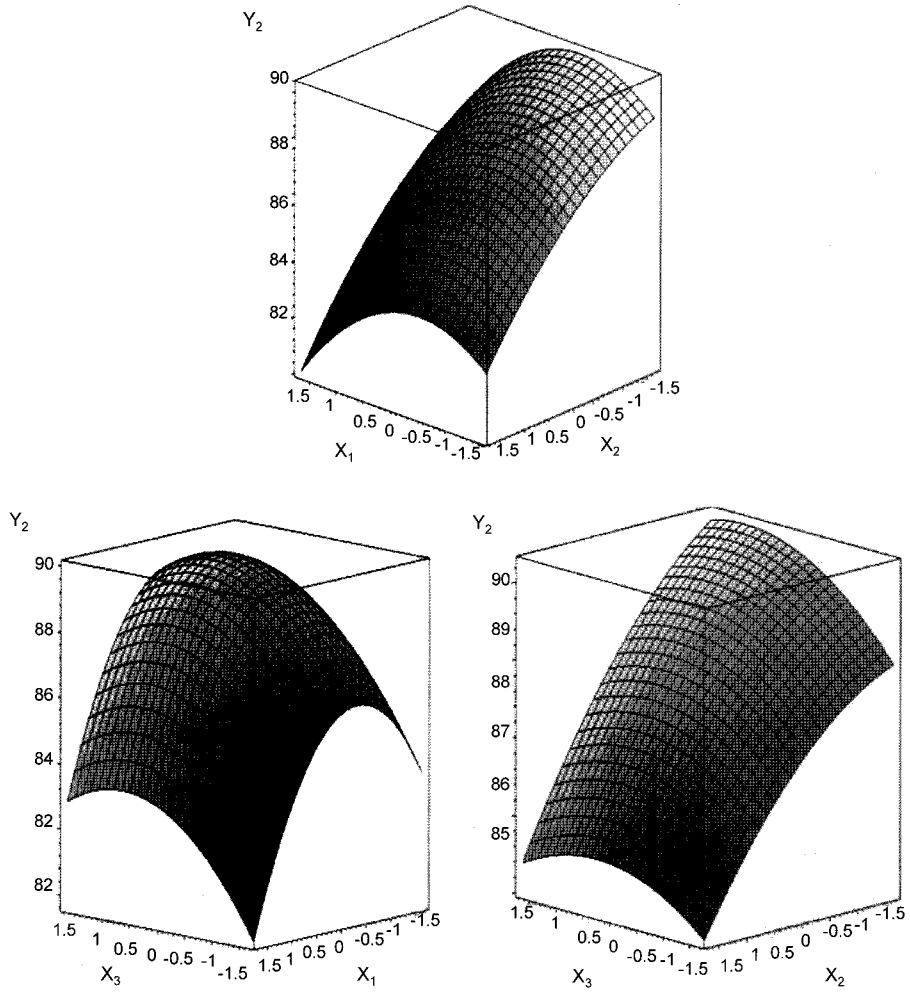

Fig. 2. Response surface plots for optimization of gelatin extraction from rockfish skin based on Y_2 (yield gelatin, %). X_1 (concentration of $Ca(OH)_2$. M), X_2 (extraction temperature, °C), X_3 (extraction time, min).

variables affected dependent variables with X_2 (extraction temperature) being the most important factor. Therefore, the setting of extraction temperature could be the key factor in gelatin processing.

Verification of the predicted value

A verification experiment was conducted under optimal conditions (concentration of $Ca(OH)_2$=1.1 M, extraction temperature=106.6°C, extraction time=68.8 min) to compare the predicted values (gelatin content=87.8%, yield of gelatin=19.1%) and the actual values of dependent variables, the results are shown in Table 8. Values of gelatin prepared under optimal conditions were 87.5% for gelatin content and 18.9% for yield. No significant differences were observed between the actual and predicted values. These results suggested that the estimated RSM was suitable for optimizing gelatin processing from rockfish skin.

Table 8. Experimental and predicted results of verification under optimized conditions

Dependent variables	Predicted value	Experimental value
Y_1 (gelatin content, %)	87.8	87.5
Y_2 (yield, %)	19.1	18.9

Gelatin content, proximate composition, yield, and Hunter color value

Gelatin content, proximate composition, yield and Hunter color value between rockfish skin gelatins extracted under high temperature/high pressure (G-HT/HP) and ordinary temperature/time (G-OT/T) are compared in Table 9. Yield in G-HT/HP was 18.9%, which was 54% higher than that of G-OT/T (12.3%). These results were advantageous for manufacturing gelatin because the cost of the resultant gelatin could be cut by extraction at high temperature/high pressure.

Table 9. Gelatin content, proximate composition, yield and Hunter color value of rockfish skin gelatins extracted under temperature/high pressure (G-HT/HP) and ordinary temperature and time (G-OT/T)

Components		Rockfish skin gelatin[1)]	
		G-HT/HP	G-OT/T
Gelatin content (%)		87.5 ± 0.1	90.7 ± 0.30
Proximate composition (g/100 g)	Moisture	09.1 ± 0.4	9.0 ± 0.1
	Protein	91.0 ± 0.4	90.9 ± 0.20
	Lipid	10.3 ± 0.1	0.0 ± 0.0
	Ash	10.4 ± 0.1	0.5 ± 0.1
Yield of gelatin (%)		18.9 ± 0.30	12.3 ± 0.2
Hunter color value	L	48.15 ± 0.91	60.04 ± 0.43
	a	2.33 ± 0.10	2.29 ± 0.08
	b	10.72 ± 0.16	15.20 ± 0.24
	ΔE	49.81 ± 0.90	39.67 ± 0.48

[1)]G-HT/HP, gelatin extracted at 106.6°C for 68.8 min; G-OT/T, gelatin extracted at 60°C for 120 min.

Table 10. Physical properties of rockfish skin gelatins extracted under high temperature/high pressure (G-HT/HP) and ordinary temperature and time (G-OT/T)

Components	Rockfish skin gelatin[1)]	
	G-HT/HP	G-OT/T
Gel strength (g)	328.8 ± 4.3	349.9 ± 6.5
Gelling point (°C)	113 ± 0	115 ± 0
Transmission (% at 660 nm)	199.9 ± 0.1	199.7 ± 0.1

[1)]G-HT/HP, gelatin extracted at 106.6°C for 68.8 min; G-OT/T, gelatin extracted at 60°C for 120 min.

Proximate composition of G-HT/HP, 9.1% moisture, 91.0% crude protein, 0.3% crude lipid, and 0.4% crude ash, was similar to that of G-OT/T (9.0% moisture, 90.9% crude protein, 0% crude lipid, and 0.5% crude ash). Hayashi et al. (1990) reported that edible gelatin should contain less than 16% moisture and less than 3% crude ash. The gelatin content of G-HT/HP was 87.5%, which was slightly lower than that of G-OT/T (89.7%). These results suggested that the G-HT/HP extracted under high temperature/high pressure was contaminated by small amounts of non-collagenous material.

Hunter color values of G-HT/HP were 48.15 in lightness, 2.33 in redness, and 10.75 in yellowness. The Hunter color values of G-HT/HP were lower in the lightness and yellowness than those of G-OT/T, while redness was similar in both. No residual heavy metals, such as arsenic, lead, copper, zinc, and mercury, were detected in G-OT/T or G-HT/HP (data not shown).

Molecular weight distribution profile

G-HT/HP and G-OT/T were separated by gel chromatography on a Sephadex G-50 to investigate the molecular weight distribution profile; the results are shown in Fig. 3. The molecular weight distribution of gelatins varied according to extraction temperature. The absorbances of both G-OT/T and G-HT/HP were determined in fractions collected from 12 min to 21 min. G-HT/HP had two sharp peaks, and the main peaks were detected in each fraction collected from 13 min to 15 min, while G-OT/T had a broad single peak and the main peak was detected in the fraction collected at 15 min. The difference in molecular weight distribution between G-OT/T and G-HT/HP was probably due to differences in temperature and pressure for gelatin extraction.

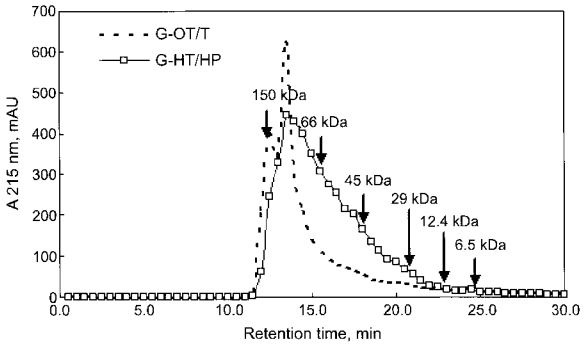

Fig. 3. Molecular weight distribution profiles of gelatin extracted under high temperature/high pressure (G-HT/HP) and gelatin extracted under ordinary condition (G-OT/T) from rockfish skin.
G-HT/HP, gelatin extracted at 106.6°C for 68.8 min; G-OT/T, gelatin extracted at 60°C for 120 min; Standards, alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), cytochrome C (12.4 kDa), aprotinin (6.5 kDa).

Physical properties

Physical properties of G-HT/HP and G-OT/T were as follows: gel strength, 328.8 g and 349.9 g, respectively; gelling point, 13°C and 15°C, respectively; and transmission, 99.9% and 99.7%, respectively. The results regarding the physical properties of rockfish skin gelatin indicated that the gel strength and gelling point of G-HT/HP were inferior to those of G-OT/T, but that transmission was comparable between the two samples. Gudmundsson (2002) reported that the gelling points of bovine and porcine gelatin were 22.6°C and 24.7°C, respectively, which were superior to those of G-HT/HP and G-OT/T rockfish skin gelatin. The physical properties, with the exception of transmission, suggested that rockfish skin gelatin is not equivalent to land animal gelatin as a resource for ordinary foods, cosmetic, pharmaceutical products, and other products, except health-

functional foods for which physical properties are not important. The differences in gel strength and gelling point between G-OT/T and G-HT/HP were probably attributable to extraction at high temperature and high pressure, which resulted in superabundant extraction of low-molecular-weight proteins from rockfish skin (Cho et al., 2005). Arnesen and Gildberg (2007) reported that gelatin extracted at high temperature has inferior gelling properties to that extracted at low temperature.

Based on physicochemical properties, G-HT/HP was unsuitable as a resource for ordinary foods, cosmetics, pharmaceutical products, and other products requiring high physical properties, but could be used as a resource for health-functional foods.

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References

AOAC. 1990. Official methods of analysis. 15thed. Washington, D.C.

Arnesen JA and Gildberg A. 2002. Preparation and characterisation of gelatine from the skin of harp seal (*Phoca groendlandica*). Bioresource Technology 82, 191-194.

Arnesen JA and Gildberg A. 2007. Preparation and characterisation of gelatine from Atlantic salmon (*Salmo salar*). Bioresource Technology 98, 53-57.

Bligh EG and Dyer WJ. 1959. A rapid method of lipid extraction and purification. Can J Biochem Physiol 37, 911-917.

Box GEP and Wilson KB. 1951. On the experimental attainment of optimum conditions. J Royal Stat Soc (Series B) 13, 1-45.

Cho SM, Gu YS and Kim SB. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloids 19, 221-229.

Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB and Kim SB. 2004. Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocollids 18, 573-579.

Fernandez-Diaz MD, Montero P and Gomez-Guillen MC. Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. Food Hydrocolloids 17, 281-286.

Gomez-Guillen MC and Montero P. 2001. Extraction of gelatin from megrim (*Lepidorhombus boscil*) skins with several organic acids. J Food Sci, 66, 213-216.

Gudmundsson M. 2002. Rheological properties of fish gelatins. J Food Sci 67, 2172-2176.

Gudmundsson M and Hafsteinsson H. 1997. Gelatin from cod skins as affected by chemical treatment. J Food Sci 62, 37-39.

Hamada H. 1990. Effects of the preparation conditions on the physical properties of shark-skin gelatin gels. Nippon Suisan Gakkaishi 56, 671-677.

Hayashi R, Kawamura Y, Ohtsuka T and Itoh N. 1990. Preparation of amidated gelatins and their physicochemical properties. J Agric Biol Chem 54, 2213-2218.

ISO. 1978. International Organization for Standardization ISO 3496-1978. Meat and meat products-Determination of L(-) hydroxyproline content.

Jamilah B and Harvinder KG. 2002. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*) Food Chem 77, 81-84.

KFDA (Korean Food and Drug Administration) Food Code. 2006. Moon-Yeoung Publishing Co., Seoul, Korea, p 70-72.

Kim JS. 1992. Improvement of the functional properties of fish skin gelatin and its utilization. PhD thesis. Pukyong National University, Busan, Korea.

Kim JS and Cho SY. 1996. Screening for raw material of modified gelatin in marine animal skins caught in coastal offshore water in Korea. Agric Chem Biotechnol 39, 134-139.

Kim JS, Ihm CW and Kim PH. 1996. Preparation and properties of gelatin from conger eel skin. J Agric Chem Biotechnol 39, 274-281.

Kim JS, Kim JG and Cho SY. 1997. Screening for the raw material of gelatin from the skins of some pelagic fishes and squid. J Korean Fish Soc 30, 55-61.

Kim JS and Park JW. 2004. Characterization of acid-soluble collagen from Pacific whiting surimi processing byproducts. J Food Sci 69, C637-642.

Kim JS and Park JW. 2005. Partially purified collagen from refiner discharge of Pacific whiting surimi processing. J Food Sci 70, C511-516.

Kim SK, Byun HG, Park PJ and Shahidi F. 2001. Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. J Agric Food Chem 49, 2992-2997.

Kolodziejska I, Skierka E, Sadowska M, Kolodziejski W and Niecikowska C. 2008. Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem 107, 700-706.

Mendis E, Rajapakse N and Kim SK. 2005. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydro-

lysate. J Agric Food Chem 53, 581-587.
Muyonga JH, Cole CGB and Duodu KG. 2004. Extraction and physico-chemical characterisation of Nile perch (*Latesniloticus*) skin and bone gelatin. Food Hydrocolloids 18, 581-592.
Pharmaceutical Society of Japan. 1980. The health experimental method notes. Kum-Won Publishing Co. Tokyo, Japan, p 728-732.
Regenstein JM, Goldhor S and Graves D. 2003. Increasing the value of Alaska pollock byproducts. Proceeding of the 2nd International Seafood By-product Conference. University of Alaska, Fairbanks, AK. pp 459-482.
Sato K, Ohashi C, Ohtsuki K and Kawabata M. 1991. Type V collagen in trout (*Salmo gardineri*) muscle and its solubility change during chilled storage of muscle. J Agric Food Chem 39, 1222-1225.
Tsutagawa Y, Hosogai Y and Kawai H. 1994. Comparison of mineral and phosphorus contents of muscle and bone in the wild and cultured horse mackerel. J Food Hyg Soc Japan 34, 315-318.
Yang H, Wang Y, Jiang M, Oh JH, Herring J and Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J Food Sci 72, C188-194.
Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci 69, 393-398.

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