Physicochemical characteristics and processing optimization of gelatin from dorsal skin of yellowfin tuna (*Thunnus albacares*)

황다랑어 (*Thunnus albacares*) 등껍질을 이용한 젤라틴의 제조 최적화 및 물리화학적 특성

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Physicochemical characteristics and processing optimization of gelatin from dorsal skin of yellowfin tuna (*Thunnus albacares*)

A Dissertation

by

Seung-Mock Cho

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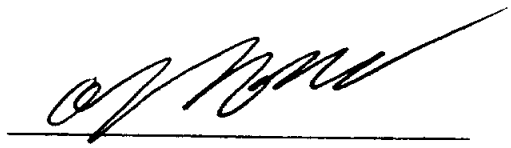

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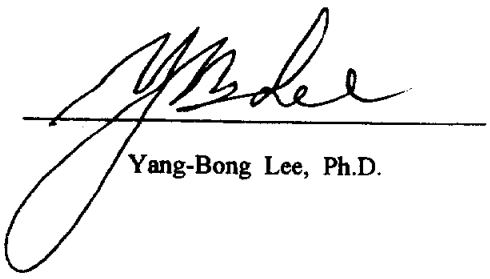

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Physicochemical characteristics and processing optimization of gelatin from dorsal skin of yellowfin tuna (*Thunnus albacares*)

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Abstract

This work is to optimize gelatin processing from dorsal skin of yellowfin tuna (*Thunnus albacares*) using response surface methodology, and to compare physical properties of yellowfin tuna skin gelatin with those of bovine and porcine skin gelatins. Central composite design was adopted in gelatin processing for processing optimization. Concentration of NaOH (X_1), treatment time (X_2), extraction temperature (X_3) and extraction time (X_4) were chosen for independent variables. Dependent variables were gel strength (Y_1) and gelatin content (Y_2). Optimal conditions were X_1 = 1.89

(%), X_2 = 2.87 (days), X_3 = 58.15 (°C) and X_4 = 4.72 (hrs), and predicted values of multiple response optimal conditions were Y_1 = 429.1 (Bloom) and Y_2 = 89.7 (%). In order to investigate physicochemical characteristics of yellowfin tuna skin gelatin, proximate components, gel strength, gelling and melting points, amino acid composition, pH, SDS-pattern and dynamic viscoelastic properties were measured. The effects of gelatin concentration, maturation time, heat and freeze treatment on the gel strength of yellowfin tuna skin gelatin were studied. The gel strength of yellowfin tuna skin gelatin (426 Bloom) was higher than bovine and porcine gelatins (216 and 295 Bloom, respectively), while gelling and melting points were lower. Viscoelastic properties of yellowfin tuna skin gelatin did not change at 20°C, but increase at 10°C as a similar pattern with mammalian gelatins. The amounts of α-chains, β- and γ-components of yellowfin tuna skin gelatin were higher than those of two mammalian gelatins. Yellowfin tuna skin gelatin showed the lower contents of proline and hydroxyproline. But, the contents of glycine, alanine and lysine of yellowfin tuna skin gelatin were the highest. The gel strengths of all gelatins were proportional to the concentration of gelatin, and yellowfin tuna skin gelatin showed the great rate of gel strength. Yellowfin tuna skin gelatin required more maturation time than two mammalian gelatins to form a firm gel. In the case of freeze treatment, the gel strength of bovine gelatin decreased slightly as the freezing times, whereas yellowfin tuna and porcine gelatins showed the similar decrease rate of gel strength.

황다랑어 (*Thunnus albacares*) 등껍질을 이용한 젤라틴의 제조 최적화 및 물리화학적 특성

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요 약

본 연구는 황다랑어 (*Thunnus albacares*) 등껍질로부터 반응표면분석이라는 통계기법을 이용하여 젤라틴의 제조 조건을 최적화하고 황다랑어 등껍질 젤라틴의 물리화학적 특성을 육상동물 유래의 젤라틴과 비교하는데 그 목적이 있다. 제조 조건의 최적화에는 중심합성계획법이 사용되었으며 독립변수로는 수산화나트륨 농도 (X_1), 처리 시간 (X_2), 추출 시간 (X_3) 및 추출 온도 (X_4), 종속변수로는 겔강도 (Y_1) 및 젤라틴 함량 (Y_2) 이 각각 선택되었다. 다중반응최적화에 의해 산출된 독립변수의 최적화 조건은 수산화나트륨 농도 (X_1) = 1.89%, 처리 시간 (X_2) = 2.87 days, 추출 시간 (X_3) = 58.15°C 및 추출 온도 (X_4) = 4.72 hrs 였으며, 최적 조건에서 예상되는 독립변수의 최대값은 겔강도 (Y_1) = 429.1

(Bloom) 및 젤라틴 함량 (Y_2) = 89.7 (%) 였다. 황다랑어 등껍질 젤라틴의 물리화학적 특성을 육상동물인 소와 돼지의 껍질에서 추출된 젤라틴들과 비교한 결과, 젤라틴의 가장 중요한 특성중의 하나인 겔강도의 경우 황다랑어 등껍질 젤라틴 (426 Bloom) 이 소 (216 Bloom) 및 돼지 (295 Bloom) 껍질 젤라틴 보다 높게 나타났다. 반면에 겔형성온도 및 녹는점은 황다랑어 등껍질 젤라틴이 육상동물 유래 젤라틴에 비해 낮게 나타났다. 육상동물 유래 젤라틴은 20°C 에서 점탄성이 증가하며 겔을 형성하였지만 황다랑어 등껍질 젤라틴은 겔을 형성하지 못하였으며 10°C에서 점탄성의 증가를 나타내었다. 황다랑어 등껍질 젤라틴의 일반 조성은 시약급의 육상동물 젤라틴과 유사하였다. 전기영동 패턴을 비교한 결과, 황다랑어 등껍질 젤라틴은 α-chain, β- 및 γ-component 의 함량이 육상동물 젤라틴에 비해 많았다. 아미노산 조성의 경우는 일반적인 어류 젤라틴의 조성과 마찬가지로 황다랑어 등껍질 젤라틴은 가장 낮은 이미노산 (proline 및 hydroxyproline) 의 함량을 보였으나 glycine, alanine 및 lysine의 함량은 가장 높았다. 모든 젤라틴들은 농도에 대하여 겔강도가 비례적으로 증가하였으며 황다랑어 등껍질 젤라틴의 경우 가장 큰 증가율을 보였다. 또한 겔강도는 숙성시간에 대해서도 모두 비례적으로 증가하였으며 황다랑어 등껍질 젤라틴은 겔이 형성되는데 육상동물 젤라틴에 비해 긴 시간을 요구하는 것으로 나타났다. 황다랑어 등껍질 젤라틴은 가열에 의해 가장 큰 겔강도의 감소를 보여 열에 대한 안정성이 매우 떨어졌다. 반면에 동결에 대한 영향을 알아본 결과, 황다랑어 등껍질 및 돼지 껍질 젤라틴은 서로 유사한 겔강도의 감소를 보였지만, 소 껍질 젤라틴의 경우 가장 낮은 감소율을 나타내었다.

Introduction

Gelatin is a gelling protein, which has widely been applied in the food and pharmaceutical industries. According to the report of the Gelatin Manufacturers of Europe (Reference), most of commercial gelatin (95%) is made from hide of porcine and bovine and the remaining part (5%) comes from bones of porcine and bovine. By-products of poultry and fish are rarely used as a resource of gelatin. The amount of gelatin used in the worldwide food industry is increasing annually (Montero & Gómez-Guillén, 2000). However, frequent occurrences of bovine spongiform encephalopathy (BSE) and foot/mouth diseases have been problems for human health and thus by-products of mammalians are limited in utility of processing in functional food, cosmetic and pharmaceutical products. Therefore, the study of gelatin from fish by-products, such as skin and bone, has increased for the replacement of mammalian resources (Gudmundsson, 2002). A few fish gelatins are available commercially, but fish gelatin is not commonly utilized because it is inferior to mammalian gelatin in rheological properties, which affect product quality (Choi & Regenstein, 2000).

In order to be applied to food and pharmaceutical industries, fish gelatin must possess the following characteristics. First, a large quantity of by-product and its economical collection are essential to be continuously

produced in industry. Second, gelatin from fish by-products must have rheological properties (gel strength, gelling and melting points, etc.) at the level of mammalian gelatin. However, it is not easy for fish by-products to satisfy the above two categories because of their bed physical properties. Fish captured in large quantities, such as anchovy, are not available for resource of gelatin because they have small body and are used whole body. Also, lumpfish (Osborne et al., 1990), tilapia (Jamilah & Harvinder, 2002), conger and squid (Kim & Cho, 1996), cod, hake, megrim and sole (Gómez-Guillén et al., 2002) have been researched to produce gelatin. They have the advantage of mass by-products such as skin, but show limit of industrial utilization because of less desirable physical properties than mammalian gelatin. Gelatin from fish bone (shark cartilage) does not have also better physical properties than mammalian gelatin (Cho et al., 2004)

In the meantime, tuna (yellowfin, skipjack, bigeye etc.) is one of the worldwide favorite fish that was captured about 3,400,000 MT every year (2001 World Capture Production of FAO Fisheries Department). Specially, the tuna occupies 12% of total amount of fish production in Korea (2003 Production Database of Ministry of Maritime Affairs & Fisheries of Korea). Tuna is usually processed as canned food and sliced raw meat in a factory, and by-products of tuna are affluent and collected at once. For this reason, if physical properties of gelatin from tuna skin resemble mammalian gelatin, tuna skin can possibly be a replacement resource of mammalian gelatin.

In the present study, investigations were divided into two parts. The first part was the optimization of gelatin processing from the dorsal skin of yellowfin tuna (*Thunnus albacares*) using response surface methodology (RSM, Box & Wilson, 1951). RSM has effectiveness in the optimization and monitoring of food manufacturing processing. The basic principle of RSM is to determinate model equations that describe interrelations between the independent variables and the dependent variables (Edwards & Jutan, 1997). The second part was focused on the physicochemical characteristics of yellowfin tuna (*T. albacares*) skin gelatin compared with mammalian gelatins extracted from the skins of bovine and porcine. In order to investigate physicochemical characteristics of the gelatin, proximate components, amino acid composition, pH, gel strength, gelling and melting points, SDS-pattern and dynamic viscoelastic properties were measured and compared with bovine and porcine skin gelatins.

Materials and Methods

1. Materials

Yellowfin tuna (*T. albacares*) skin was provided by Dooyoung Fisheries Co., LTD (Busan 602-030, Korea). The yellowfin tuna skin was parted into abdominal and dorsal skins, the dorsal skin of yellowfin tuna was used in this study. Proximate composition of the skin was 56.1% moisture, 6.8% crude lipid, 1.0% crude ash and 33.6% crude protein. Content of collagen, which is a precursor of gelatin, was 13.54%. Two mammalian gelatins extracted from the skin of bovine (G 9382, 225 Bloom) and porcine (G 2500, 300 Bloom), were purchased from Sigma Chemical Co. All regents used in this study were analytical grade.

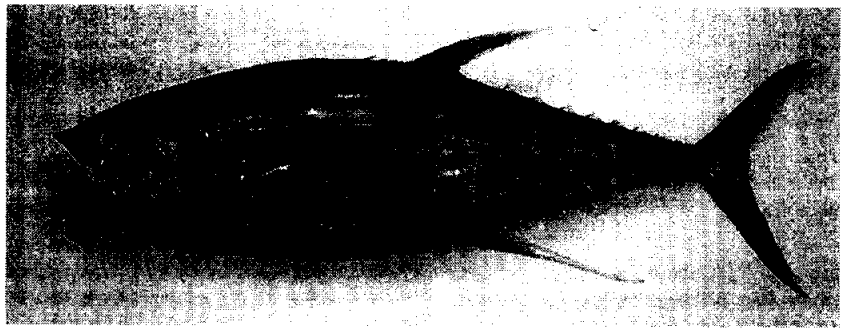

Fig. 1. A photograph of yellowfin tuna (*Thunnus albacares*).

2. Methods

2.1. Preparation of gelatin from dorsal skin

The yellowfin tuna skin was washed, chopped and frozen at −15°C until used. The cleaned skin was treated with 8 volumes (v/w) of alkali solution (1−3% NaOH) at 10°C in shaking incubator at 200 rpm (HB-201SF, Hanbaek Scientific Co., Korea) for 1−5 days to remove the non-collagen protein and subcutaneous tissue after they were swollen. After the alkali treatment, the skin was neutralized with 6 N HCl and washed. For hot-water extraction, 6 volumes of (v/w) of distilled water were added and heated at temperature ranging 40−80°C for 1−9 hrs. The extracted solution was centrifuged for 30 min at 900×g at 30°C. The upper phase was vacuum-filtered with a filter paper (5A 110 mm, Advantec, Japan), and the filtered solution was vacuum-concentrated to 10 brix at 60°C and dried at 1.4 m/sec for 24 hrs in a hot-air dryer (WFO-601SD, EYELA, Japan).

2.2. Determination of proximate components

Moisture content (oven-drying procedure), crude protein (N×6.25), lipid (ether extraction) and ash content were estimated by the AOAC official method (AOAC, 2000). The analyses were replicated three times.

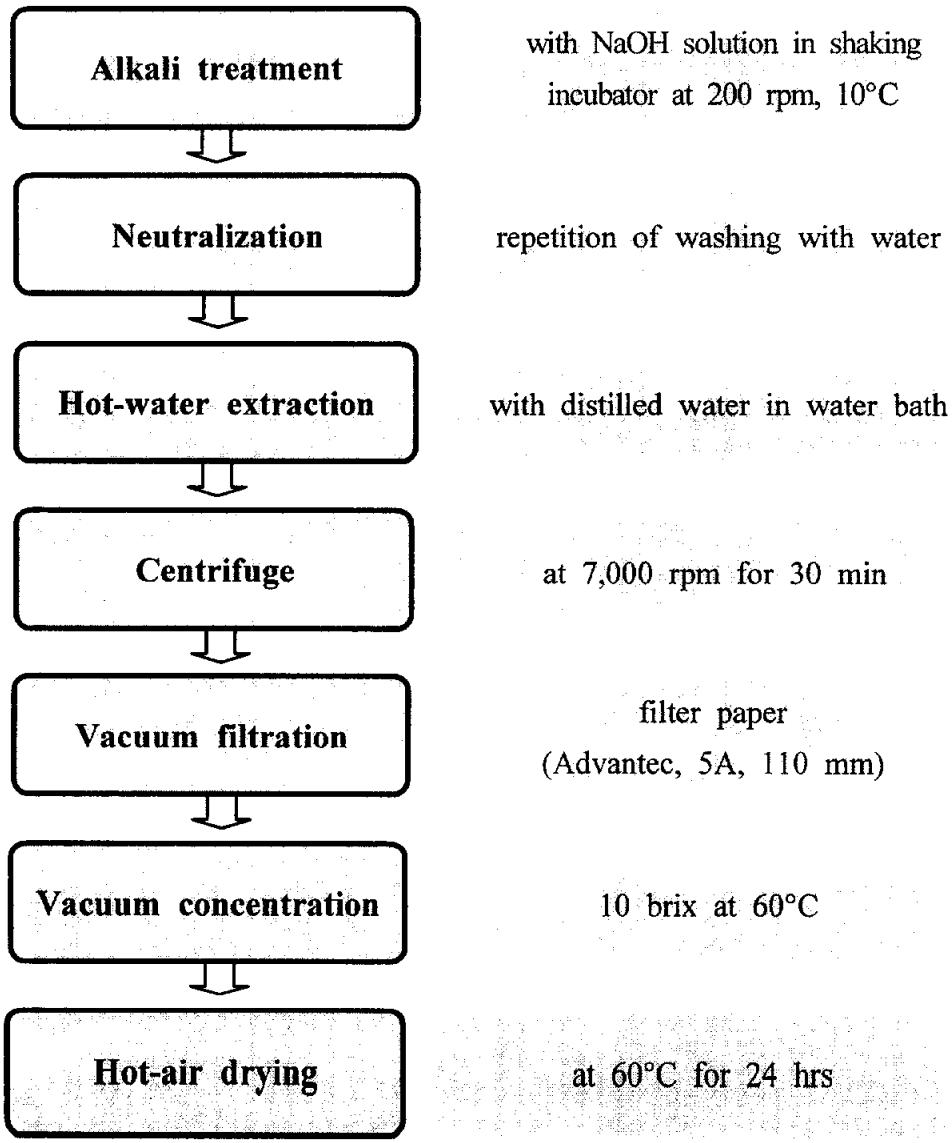

Fig. 2. Flow of gelatin processing from yellowfin tuna (*T. albacares*) skin.

2.3. Measurements of pH of gelatin solution

Gelatin pH measurement was performed by the methods of Leach & Eastoe (1977) and Choi & Regenstein (2000). A 0.1 mg gelatin was dissolved in 10 mL distilled water at 60°C, and the pH of the prepared gelatin solution was measured as a liquid solution with pH meter (Accument model 15, Fisher Scientific Co., U.S.A.) at 25°C.

2.4. Determination of gelatin content

Gelatin content was estimated by measuring hydroxyproline content by the method of Sato et al. (1991), using a conversion factor of 11.42. Hydroxyproline content was determined by the method described in ISO (1978), with slight modifications. Dried gelatin (100 mg) was placed into test tube, and 5 mL of 6 N HCl were added to test tubes and put in a dry bath for 12 hrs to hydrolysis. After acid hydrolysis, the sample solutions were neutralized with 6 N NaOH, mixed with 2 mL of acetate/citrate buffer and massed up 25 mL with 0.3 M NaCl. Aliquot was transferred into test tube, isopropanol (300 μL) and oxidant solution (600 μ L) were added and let it sit at room temperature for 4 min. After 4 min, Ehrlichs reagent solution (4 mL) was added to each tube, mixed, and heated for 25 min in water bath at 60°C. Absorbance of the solutions was

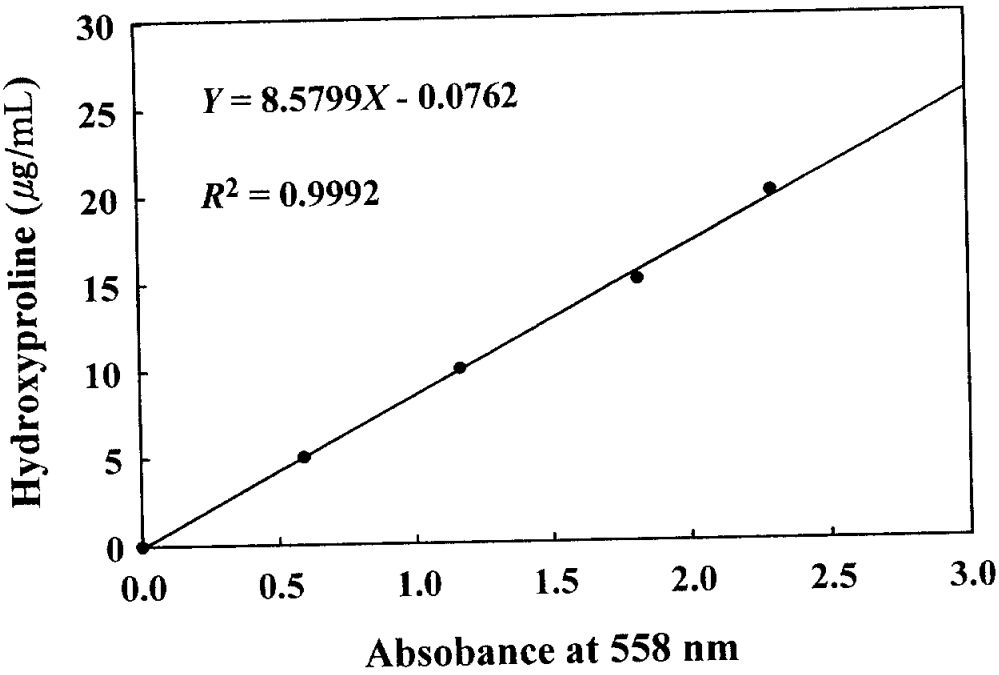

Fig. 3. Calibration curve for the determination of hydroxyproline content.

measured with a spectrophotometer (UV-140-02, Shimadzu, Japan) at 660 nm. The hydroxyproline content of the sample solutions was calculated from a calibration curve (Fig. 3) derived from standard using analytical grade hydroxyproline purchased from Sigma Chemical Co.

2.5. Amino acid analysis

Dried gelatin sample (5 mg) was dissolved in 3 mL of 6N HCl and heated in vacuum-sealed glass tubes at 110°C for 24 hrs using in a dry bath (Dry bath incubator 11-718-2, Fisher Scientific Co., U.S.A.). After acid hydrolysis, samples were vacuum-dried, dissolved in citric acid buffer (pH 2.2, Sigma Chemical Co.) and injected into an amino acid auto analyzer (Amino acid analyzer S-433H, Sycam, Germany).

2.6. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed as the method of Laemmli (1970) using a Mini-Protean 3 (Bio-Rad Laboratories, Hercules, CA). Polyacrylamide gel was prepared with 4% stacking gel and 5% resolving gel. Three gelatin samples and type I collagen from the bovine skin, were dissolved 5 mg/mL in distilled water at 60°C. The prepared gelatin solutions were mixed with 0.5 M Tris-HCl buffer (pH 6.8) containing 5% 2-mercaptoethanol, 10% SDS, 20% glycerol and 0.1% bromophenol blue, and heated at 90°C for 5 min.

Sample solutions were loaded onto each gel and electrophoresed at 25 mA/gel in slab gels. Protein bands were stained for 2 hrs with 0.25% (w/v) Coomassie brilliant blue R250. Type I collagen from the bovine skin was used as markers of α-chains, β- and γ-component mobilities.

2.7. Determination of gel strength

Gel strength was determined according to the AOAC official method 948.21 (AOAC, 2000), using rheometry (Compac-100, Sun Scientific Co. LTD., Japan). Gelatin was dissolved with distilled water (6.67%, w/v) at 60°C for 30 min until completely dispersed and then kept at 7°C for 17 hrs. After cool maturation the gel strength, expressed in Bloom value, was measured with the following conditions; plunger, 12.7 mm diameter; penetration depth, 4 mm; penetration speed, 2 cm/min.

2.8. Measurement of dynamic viscoelastic properties

Dynamic viscoelastic properties were measured by a concentric cylinder geometry of the rheometer (Rheostress 1 RS30, HAAKE Co., Ltd., Germany). The gelatin solution (6.67%, w/v) was prepared in distilled water at 60°C. The measurement was performed at a scan rate of 0.5 °C/min, frequency 1 Hz, oscillating applied stress 3 Pa and gap 4.2 mm, while temperature was cooled from 40°C to 5°C and heated from 5°C to

40°C. The elastic modulus (G'; Pa), the loss modulus (G"; Pa) and the phase angle (rad) were plotted as a function of temperature. In order to study for the effect of time, viscoelastic properties were measured for 1 and 3 hrs at constant temperature (20 and 10°C).

2.9. Determination of gelling and melting points

Gelling and melting points were determined as the method of Gudmundsson (2002). The gelling point was evaluated from the intersection point where the elastic modulus (G', kPa) and the loss modulus (G", kPa) during the cooling process. The melting point was done during the heating process in a same manner as for the gelling point.

2.10. Experimental design

Central composite design (CCD, Box & Wilson, 1951) was adopted in the optimization of gelatin processing from the dorsal skin of yellowfin tuna. CCD in the experimental design consists of 24 factorial points, eight axial points (α=2) and three replicates of the central point (Table 2). Processing of gelatin included two important processes, alkali treatment and hot-water extraction. Concentration of NaOH (%, X_1) and treatment time (days, X_2) in alkali treatment and extraction temperature (°C, X_3) and extraction time (hrs, X_4) in hot-water extraction were chosen for

independent variables. The range and center point values of four independent variables were based on the results of preliminary experiments (Table 1). Gel strength (Bloom, Y_1) and gelatin content (%, Y_2) were selected as the dependent variables for the combination of the independent variables were given in Table 2. Experimental runs were randomized in order to minimize the effects of unexpected variability in the observed responses.

2.11. Analysis of data

The response surface regression (RSREG) procedure of the Statistical Analysis System software (Version 8.01, SAS Institute Inc., U.S.A.) was used to fit the following quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j$$

where Y is the independent variable (gel strength and gelatin content), β_0 is constant, β_i, β_{ii}, β_{ij} are regression coefficients and X_i, X_j are levels of the independent variables. Ridge Max option of RSREG procedure was used to compute the estimated ridge of optimum response for increasing radii from the center of the origin design. Multiple response optimization was heuristically calculated by desirability function of MINITAB statistical software (Version 13, Minitab Inc., U.S.A.), in order to search for the condition

Table 1. Experimental range and values of the independent process variables in the central composite design for gelatin processing from yellowfin tuna (*T. albacares*) skin

Independent variables	Symbol	Range and levels				
		-2	-1	0	1	2
Concentration of NaOH (%)	X_1	1	1.5	2	2.5	3
Treatment time (days)	X_2	1	2	3	4	5
Extraction temperature (°C)	X_3	40	50	60	70	80
Extraction time (hrs)	X_4	1	3	5	7	9

Table 2. Central composite design and responses of the dependent variables for gelatin processing from yellowfin tuna (*T. albacares*) skin to the independent variables

Run No.	Coded levels of variable				Response	
	X_1	X_2	X_3	X_4	Y_1	Y_2
1	−1	−1	−1	−1	255	85.0
2	−1	−1	−1	+1	267	83.8
3	−1	−1	+1	−1	219	82.3
4	−1	−1	+1	+1	119	81.1
5	−1	+1	−1	−1	210	84.9
6	−1	+1	−1	+1	222	83.8
7	−1	+1	+1	−1	174	82.2
8	−1	+1	+1	+1	100	81.7
9	+1	−1	−1	−1	199	84.7
10	+1	−1	−1	+1	211	83.5
11	+1	−1	+1	−1	163	81.9
12	+1	−1	+1	+1	100	80.7
13	+1	+1	−1	−1	152	84.5
14	+1	+1	−1	+1	164	83.3
15	+1	+1	+1	−1	116	81.7
16	+1	+1	+1	+1	100	80.5
17	−2	0	0	0	296	82.8
18	+2	0	0	0	172	82.1
19	0	−2	0	0	205	80.4
20	0	+2	0	0	130	80.2
21	0	0	−2	0	150	86.9
22	0	0	+2	0	120	81.3
23	0	0	0	−2	240	85.5
24	0	0	0	+2	190	83.2
25	0	0	0	0	422	89.5
26	0	0	0	0	424	89.6
27	0	0	0	0	418	89.3

simultaneously satisfying two dependent variables (Y_1 and Y_2). The response surface plots were developed using Maple software (Maple 7, Waterloo Maple Inc., Canada) and represented a function of two independent variables while keeping the other two independent variables at the optimal values.

2.12. Statistical treatment

All experiments were analyzed with three repetitions per sample using one-way analysis of variance (ANOVA) ($P<0.05$). Means were separated using Duncan's multiple range test ($\alpha = 0.05$). Regression analysis for the gel strength as function of gelatin concentration and maturation time was performed by REG procedure of SAS software (Version 8.01, SAS Institute Inc., U.S.A.).

Results and Discussion

1. Optimization of gelatin processing from tuna dorsal skin

1.1. Diagnostic checking of the fitted model

The RSREG procedure for SAS software was employed to fit the quadratic polynomial equation to the experimental data. All the coefficients of linear (X_1, X_2, X_3, X_4), quadratic (X_{11}, X_{22}, X_{33}, X_{44}) and interaction were calculated for significance with t-statistic and the estimated coefficients of each model are presented in Table 3 and 4. All the quadratic coefficients were highly significant ($P < 0.01$) in all models. On the other hand, all the interaction coefficients except the X_3X_4 term of Y_1 (gel strength, Bloom) were not significant ($P > 0.05$). The X_2 term of Y_2 (gelatin content, %) was not significant in case of linear coefficients and the other linear coefficients were significant. In order to develop the fitted response surface model equations, all insignificant terms ($P > 0.05$) were eliminated and the fitted models are shown in Table 5. All the independent variables, X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C) and X_4 (extraction time, hrs) have negative linear, quadratic and interaction effects in two response surface models (Y_1 and Y_2).

Table 3. Estimated coefficients of the fitted quadratic polynomial equation for the response of Y_1 (gel strength, Bloom) based on t-statistic

Parameter	Parameter estimate	Standard error	T-value	P-value
Intercept	421.3333	13.7480	30.65	0.0001
X_1	-25.3333	4.8606	-5.21	0.0002
X_2	-18.6667	4.8606	-3.84	0.0024
X_3	-27.0833	4.8606	-5.57	0.0001
X_4	-12.9167	4.8606	-2.66	0.0209
X_1X_1	-49.1875	5.1555	-9.54	0.0001
X_1X_2	0.6250	5.9530	0.10	0.9181
X_1X_3	6.0000	5.9530	1.01	0.3334
X_1X_4	6.0000	5.9530	1.01	0.3334
X_2X_2	-65.9375	5.1555	-12.79	0.0001
X_2X_3	4.5000	5.9530	0.76	0.4643
X_2X_4	4.5000	5.9530	0.76	0.4643
X_3X_3	-74.1875	5.1555	-14.39	0.0001
X_3X_4	-18.6250	5.9530	-3.13	0.0087
X_4X_4	-54.9375	5.1555	-10.46	0.0001

X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

Table 4. Estimated coefficients of the fitted quadratic polynomial equation for the response of Y_2 (gelatin content, %) based on t-statistic

Parameter	Parameter estimate	Standard error	T-value	P-value
Intercept	89.4666	0.0829	1,079.01	0.0001
X_1	-0.2250	0.0293	-7.68	0.0001
X_2	-0.0333	0.0293	-1.14	0.2777
X_3	-1.3583	0.0293	-46.34	0.0001
X_4	-0.5583	0.0293	-19.05	0.0001
X_1X_1	-1.7458	0.0310	-56.15	0.0001
X_1X_2	-0.0750	0.0359	-2.09	0.0587
X_1X_3	-0.6250	0.0359	-1.74	0.1073
X_1X_4	-0.0500	0.0359	-1.39	0.1890
X_2X_2	-2.2833	0.0310	-73.43	0.0001
X_2X_3	0.0375	0.0359	1.04	0.3169
X_2X_4	0.0500	0.0359	1.39	0.1890
X_3X_3	-1.3333	0.0310	-42.88	0.0001
X_3X_4	0.0375	0.0359	1.04	0.3169
X_4X_4	-1.2708	0.0310	-40.87	0.0001

X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

Table 5. Response surface model for processing conditions of gelatin from yellowfin tuna (*T. albacares*) skin

Responses	Quadratic polynomial model	R^2	*P*-value
Gel strength (Bloom)	$Y_1 = 421.333 - 25.333X_1 - 18.667X_2 - 27.083X_3 - 12.917X_4 - 49.186X_1^2 - 65.937X_2^2 - 74.186X_3^2 - 53.938X_4^2 - 18.813X_3X_4$	0.9704	0.0001
Gelatin content (%)	$Y_2 = 89.467 - 0.225X_1 - 1.358X_3 - 0.558X_4 - 1.745X_1^2 - 2.283X_2^2 - 74.031X_3^2 - 1.270X_4^2$	0.9987	0.0001

X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

The coefficients of determination (R^2) for Y_1 and Y_2 were 0.9704 and 0.9987, respectively, which indicates that the model is suitable to represent the real relationships among the selected reaction parameters. The values of R^2 for all models were extremely high for response surface and significant at P = 0.01. The reason why the values of R^2 are quite high is that the experimental design was based on the adequately performed preliminary test.

1.2. Analysis of variance

The statistical significance of the quadratic polynomial model equation was evaluated by the analysis of variance (ANOVA). Table 6 and 7 shows ANOVA for the models that explain the response of two dependent variables, Y_1 (gel strength) and Y_2 (gelatin content). Cross-product terms for all the dependent variables (Y_1 and Y_2) were not significant (P = 0.1204 and P = 0.1102, respectively) at 95% probability level, whereas linear term (X_1, X_2, X_3, X_4), quadratic term (X_{11}, X_{22}, X_{33}, X_{44}) and total regression model were highly significant (P < 0.01) at 99% probability level. As the results of the lack-of-fit test, which indicates the fitness of the model, the dependent variable Y_1 was significant at a 95% probability level. However, the lack-of-fit test of Y_2 did not show a significant P-value (P = 0.6482) at a 95% probability level. The check of model adequacy was performed by a normality test (Anderson-Darling normality test) for error terms using

Table 6. Analysis of variance (ANOVA) for response of the dependent variable (Y_1, gel strength)

Sources	DF	SS	MS	F-value	P-value
Regression					
Linear	4	45,374.00	12,343.50	20.01	0.0001
Quadratic	4	170,190.00	42,547.50	75.04	0.0001
Cross-product	6	7,356.50	1,226.08	2.16	0.1204
Total model	14	222,920.00	15,922.86	28.08	0.0001
Residual					
Lack of fit	10	6,785.67	567.03	72.70	0.0136
Pure error	2	18.67	9.33	-	-
Total error	12	6,804.33	678.57	-	-
Total	26	229,724.33	16,487.98	-	-
Factors					
X_1	5	68,175.00	13,635.00	24.05	0.0001
X_2	5	101,769.00	20,354.00	35.90	0.0001
X_3	5	141,468.00	28,294.00	49.90	0.0001
X_4	5	72,519.00	14,504.00	25.58	0.0001

DF (Degrees of Freedom), SS (Sum of Square), MS (Mean Square).
X_1 (concentration of NaOH, %), X_2 (treatment time, days),
X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

Table 7. Analysis of variance (ANOVA) for response of the dependent variable (Y_2, gelatin content)

Sources	DF	SS	MS	*F*-value	*P*-value
Regression					
Linear	4	53.01	13.25	642.48	0.0001
Quadratic	4	138.27	34.57	1,675.96	0.0001
Cross-product	6	0.28	0.05	2.24	0.1102
Total model	14	191.55	13.68	663.37	0.0001
Residual					
Lack of fit	10	0.20	0.02	0.86	0.6482
Pure error	2	0.05	0.02	-	-
Total error	12	0.25	0.02	-	-
Total	26	191.80	13.70	-	-
Factors					
X_1	5	66.43	13.29	644.17	0.0001
X_2	5	111.40	22.28	1,080.27	0.0001
X_3	5	82.32	16.46	798.21	0.0001
X_4	5	42.04	8.41	407.64	0.0001

DF (Degrees of Freedom), SS (Sum of Square), MS (Mean Square).

X_1 (concentration of NaOH, %), X_2 (treatment time, days),

X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

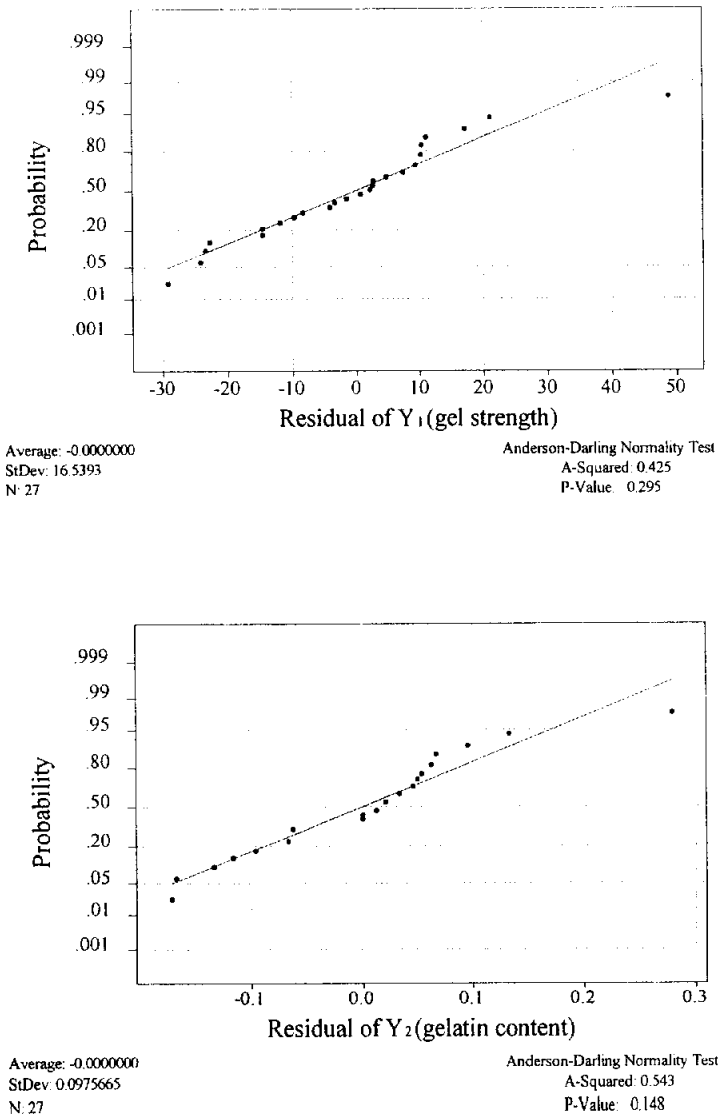

Fig. 4. Normal probability plots for error terms using residuals of the dependent variables by Anderson-Darling Normality test.

residuals of the dependent variables, Y_1 and Y_2 (Fig. 4). The error terms of two dependent variables had the normal distribution as the Anderson-Darling normality test. Therefore, response surface model represented as quadratic polynomial equation was statistically significant.

1.3. Conditions for optimum responses

Four independent variables, concentration of NaOH (2%, X_1), treatment time (3 days, X_2), extraction temperature (60°C, X_3) and extraction time (5 hrs, X_4) were chosen as the central condition of the CCD for optimizing the gelatin processing from yellowfin tuna skin. Uncoded values of independent variables were determined by the preliminary study. Optimal conditions included coded and uncoded values of each dependent variable (Y_1 and Y_2), which are shown in Table 8. According to the canonical analysis of the RSREG procedure, all the eigenvalues of Y_1 and Y_2 were negative, therefore, the stationary points were maxima. Predicted values of Y_1 and Y_2 were 429.5 (Bloom) and 89.9 (%). The gel strength is an important physical property of gelatin in a wide range of applications. In general, a high value of gel strength means good quality gelatin, so Y_1 (gel strength) and Y_2 (gelatin content) were chosen as the dependent variables. The critical values of dependent variables did almost not make a difference, but extraction temperature (54.72°C, X_3) of Y_2 (gelatin content) was lower than one of Y_1 (gel strength), because the extraction at high temperature

Table 8. Optimal conditions of gelatin processing from yellowfin tuna (*T. albacares*) skin

Dependent variables	Independent variables	Critical value		Predicted value	Stationary point
		Coded	Uncoded		
Y_1 (gel strength, Bloom)	X_1	−0.276	1.86	429.5	Maximum
	X_2	−0.153	2.85		
	X_3	−0.185	58.15		
	X_4	−0.109	4.78		
Y_2 (gelatin content, %)	X_1	−0.052	1.97	89.9	Maximum
	X_2	−0.013	2.99		
	X_3	−0.512	54.88		
	X_4	−0.226	4.55		
Average of Y_1 and Y_2	X_1	−0.164	1.92	–	–
	X_2	−0.152	2.92		
	X_3	−0.185	56.52		
	X_4	−0.107	4.67		
Multiple response optimization	X_1	−0.216	1.89	–	–
	X_2	−0.128	2.87		
	X_3	−0.185	58.15		
	X_4	−0.138	4.72		

X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

derived superabundant extraction of low-molecular proteins from tuna skin. In order to optimize two dependent variables (Y_1 and Y_2) simultaneously, desirability function of MINITAB statistical software were defined as follow conditions; goal (maximize), target (Y_1 = 429.5 and Y_2 = 89.9). Coded values of the independent variables were concentration of NaOH, X_1 = −0.219; treatment time, X_2 = −0.128; extraction temperature, X_3 = −0.185; and extraction time, X_4 = −0.138; respectively. Critical values of multiple response optimization by desirability function of MINITAB and average of Y_1 and Y_2 were nearly similar. Actual values of independent variables against coded values were X_1 = 1.89 (%), X_2 = 2.87 (days), X_3 = 58.15 (°C) and X_4 = 4.72 (hrs), respectively. Predicted values of multiple response optimal conditions were Y_1 = 429.1 (Bloom) and Y_2 = 89.7 (%) with 0.98 of the value of desirability function.

1.4. Response surface plots

Fig. 5 and 6 show the estimated response function and the effect of the independent variables (X_1, X_2, X_3, X_4) on the dependent variables (Y_1 and Y_2). As the gelatin processing has been stated in the previous experimental design section, gelatin processing needs two important processes, alkali treatment and hot-water extraction. Two independent variables of X_1 (concentration of NaOH) and X_2 (treatment time) are major factors for alkali treatment, and the other two independent variables of X_3 (extraction

temperature) and X_4 (extraction time) are major factors for hot-water extraction. Therefore, response surface plot presents interrelationship between two independent variables and one dependent variable while keeping the other two independent variables at the optimal values. Fig. 5 A and B depict the effect of independent variables on Y_1 (gel strength). As the coded values of four independent variables were closed to zero, gel strength increased. The effect of four factors was statistically significant with the factor X_3 (extraction temperature) having the higher effect. Gel strength decreased with an increase in extraction temperature from 0 (60°C) to 1.5 (75°C), because higher extraction temperature caused protein degradation, thereby producing protein fragments and lowering gelling ability (Ledward, 1986; Normand et al., 2000). However, the effect of independent variables on Y_2 (gelatin content) was different from the other dependent variables (Fig. 6 A and B). As the X_3 (extraction temperature) was increased from 0 (60°C) to 1.5 (75°C), Y_2 (gelatin content) decreased greatly. This decreased gelatin content was attributed to extraction of other low-molecular proteins at higher extraction temperature over 60°C. When considering four response surface plots, all the independent variables affected dependent variables with X_3 (extraction temperature) being the most important factor. Therefore, the setting of extraction temperature could be the key factor on gelatin processing.

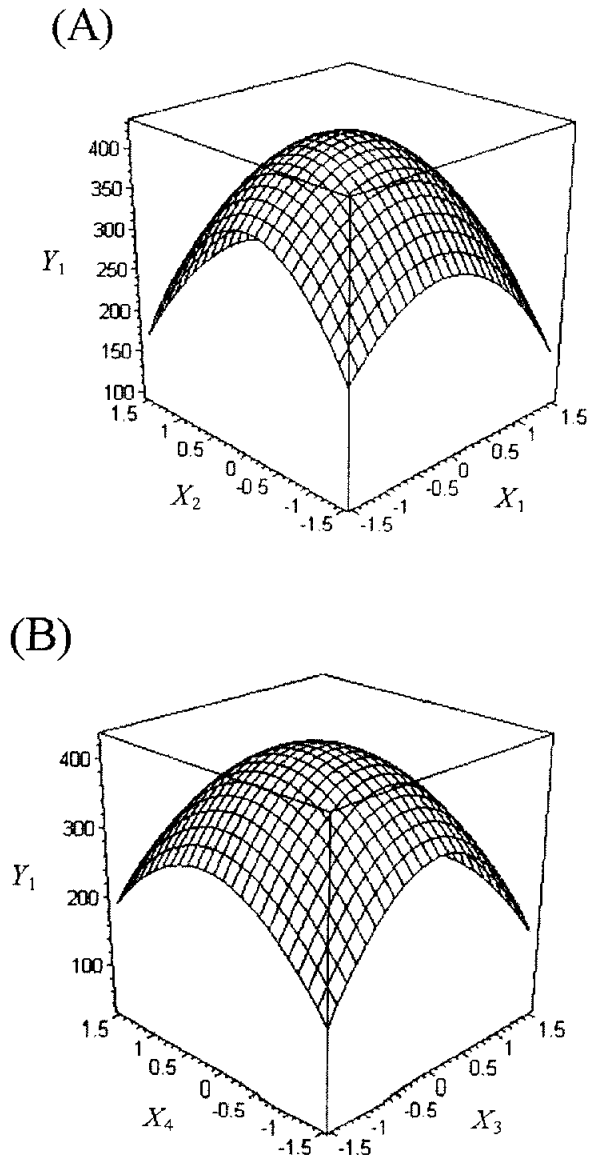

Fig. 5. Response surface plots for optimization of gelatin processing from yellowfin tuna (*T. albacares*) skin. Y_1 (gel strength, Bloom), X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

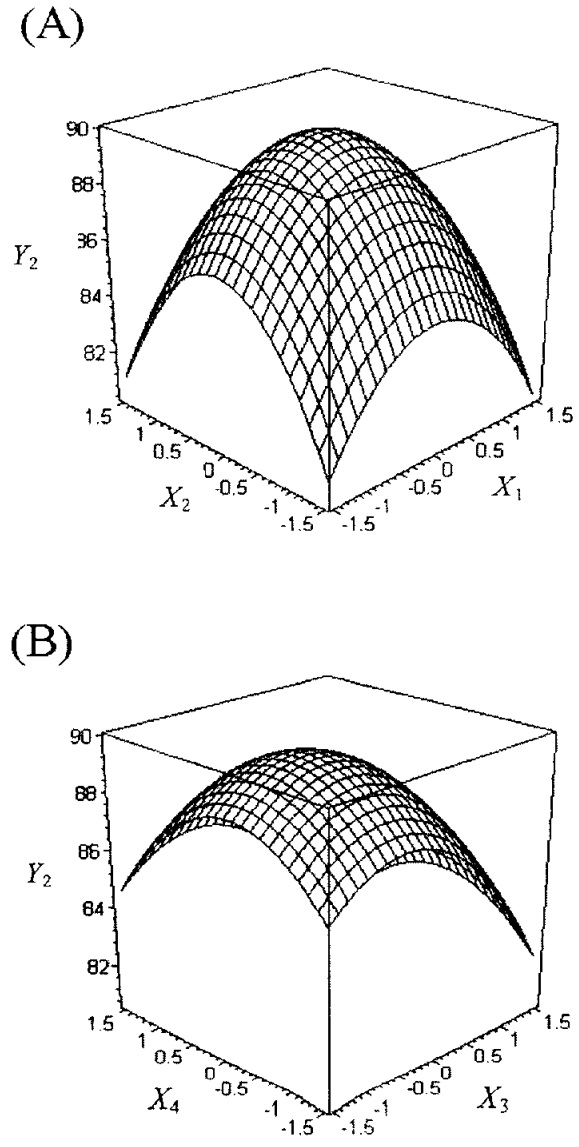

Fig. 6. Response surface plots for optimization of gelatin processing from yellowfin tuna (*T. albacares*) skin. Y_2 (gelatin content, %), X_1 (concentration of NaOH, %), X_2 (treatment time, days), X_3 (extraction temperature, °C), X_4 (extraction time, hrs).

Table 9. Experimental and predicted results of verification under optimized conditions

Dependent variables	Predicted value	Experimental value
Y_1 (gel strength, Bloom)	429.1	425.9±0.4
Y_2 (gelatin content, %)	89.7	89.7±0.1

Optimized conditions: concentration of NaOH = 1.9%, treatment time = 2.9 days, extraction temperature = 58°C, extraction time = 4.7 hrs.

1.5. Verification of predicted values

Verification experiments were conducted under optimal conditions (concentration of NaOH = 1.9%, treatment time = 2.9 days, extraction temperature = 58°C and extraction time = 4.7 hrs) to compare predicted values and actual values of dependent variables (Table 9). Actual values repeated three times were gel strength = 425.9 (Bloom) and gelatin content = 89.7 (%) against predicted values, gel strength = 429.1 (Bloom) and gelatin content = 89.7 (%). Both actual values and predicted values almost coincided each other. Therefore, the estimated response surface model was adapted for optimization of gelatin processing from yellowfin tuna skin.

2. Physicochemical characteristics of tuna dorsal skin gelatin

2.1. Proximate composition

Table 10 shows a comparison of proximate composition and pH of yellowfin tuna skin gelatin with two mammalian gelatins. Yellowfin tuna skin gelatin had a lower moisture content than bovine and porcine gelatins, but the difference of them was not significantly great. The content of crude protein (almost gelatin) of yellowfin tuna, bovine and porcine gelatins was 90.4%, 90.6% and 90.7%, respectively. Gelatin almost consists of protein and water, and impurity contents such as ash are important for quality of gelatin. According to food (Gelatin, FCC, 1994) and pharmaceutical (Gelatin, USP XXIII NF 18, 1994) standards of United States of America for gelatin, their maximum ash contents were 3% and 2%, respectively. Ash content of yellowfin tuna skin gelatin was 0.83%, and much lower than criteria of those. In accordance with product information supplied by Sigma, the pH value of bovine gelatin (type B) ranged from 5.0 to 7.5, and that of porcine gelatin (type A) ranged from pH 3.8 to pH 5.5. The pH values of bovine and porcine gelatins, in this study, were pH 5.9 and pH 4.7, respectively. The pH value of yellowfin tuna skin gelatin was pH 6.5, and included in the range of bovine gelatin.

Table 10. Proximate composition and pH of yellowfin tuna (*T. albacares*), bovine and porcine skin gelatins

	Gelatin type		
	Yellowfin tuna	Bovine	Porcine
Moisture (%)	8.2±0.3	8.9±0.1	8.5±0.3
Crude lipid (%)	1.0±0.2	0.2±0.2	0.9±0.1
Crude ash (%)	0.8±0.3	0.9±0.2	0.5±0.2
Crude protein (%)	90.4±0.2	90.6±0.2	90.9±0.2
pH	6.5	5.9	4.7

2.2. Gel strength, gelling and melting points

Gel strength is one of the important properties of gelatin, and the purpose of gelatin was determined by the range of gel strength values. Generally, fish gelatin has lower gel strength than mammalian gelatin (Norland, 1990). Especially, characteristics of collagen have influence on the physical properties of gelatin, because gelatin is derived from collagen. Gómez-Guillén et al. (2002) reported that tropical-fish, such as tilapia, was a superior material for gelatin processing (Grossman & Bergman, 1992), however, cold-water fish, cod gelatin has poorer physical properties (Gudmundsson & Hafsteinsson, 1997). The gel strength of gelatin from the dorsal skin of yellowfin, which is a tropical-fish (15−31°C), was compared with the two mammalian gelatins shown in Table 11. The gel strengths of bovine and porcine gelatins were 216 and 295 Bloom, respectively. The values of gel strength were slightly lower than the values in product information (225 Bloom and 300 Bloom, respectively). According to the results of gel strength measured by Choi & Regenstein (2000), fish gelatins showed lower gel strength than porcine skin gelatin (300 Bloom). However, yellowfin tuna skin gelatin in the present study had a 426 Bloom gel strength, which is remarkable for gel strength of fish gelatin. As the results of gelatin content, yellowfin tuna skin gelatin showed similar content (89.9%) with mammalian gelatins (90.1% and 90.7%).

Table 11. Comparison of yellowfin tuna (*T. albacares*), bovine and porcine skin gelatins

	Gelatin type		
	Yellowfin tuna	Bovine	Porcine
Gel strength (Bloom)	426±2.9	216±2.2	295±1.9
Gelling point (°C)	18.7	23.8	25.6
Melting point (°C)	24.3	33.8	36.5
Gelatin content (%)	89.9±0.2	90.1±0.1	90.7±0.1

Yellowfin tuna could, therefore, be a good resource of gelatin because of its high gelatin content and gel strength.

Bovine and porcine gelatins have considerably higher gelling and melting points than most fish gelatins, and the high gelling and melting points expand the range of gelatin application (Leuenberger, 1991; Gilsenan & Ross-Murphy, 2000; Choi & Regenstein, 2000; Gudmundsson, 2002). The gelling and melting points of yellowfin tuna skin gelatin were compared with two mammalian gelatins shown in Table. 11. As regards the gelling and melting points determined by Gudmundsson (2002), the gelling point of bovine and porcine gelatin were 22.6 and 24.7°C, respectively, and were similar with 23.8 and 25.6°C in this study. On the other hand, the melting points (33.8 and 36.5°C) in this study were higher than the results (29.7 and 32.3°C) of Gudmundsson. The reason why melting point is higher, is that the heating rate in this study was higher (0.5°C/min) than Gudmundsson's study (0.1°C/min). The gelling point (18.7°C) and melting point (24.3°C) of yellowfin tuna skin gelatin were very lower than two mammalian gelatins. This pattern of the gelling and melting points is similar with the other fish gelatins, especially tuna gelatin and tilapia (warm-water fish) (Gilsenan & Ross-Murphy, 2000; Gudmundsson, 2002).

2.3. Dynamic viscoelastic properties

The measurement of dynamic viscoelastic parameters was performed

during both cooling (from 40°C to 5°C) and heating (from 5°C to 40°C) at the rate of 0.5°C/min. Fig. 7, 8 and 9 present the change in the elastic modulus (G', kPa), the loss modulus (G", kPa) and the phase angle (rad), respectively. The elastic modulus (G', kPa) and the loss modulus (G", kPa) are indicators of the gelling ability of gelatin, and the phase angle (Fig. 9) presents a phase change of gelatin solutions. Upper and under lines indicated liquid and solid phases, respectively. Yellowfin tuna skin gelatin began to form a gel at lower G' and G" values than the two mammalian gelatins in the cooling phase. The G' value of yellowfin tuna skin gelatin increased rapidly from 10°C to 5°C (Fig. 7. A), whereas G" increased gradually (Fig. 8. A). The G' and G" values of all gelatins at 5°C of heating process were higher than those at 5°C of cooling process. The reason for this increase is that gelatin gels were continued to mature for a few minutes until the measurement restarted. During heating, the G' and G" values of mammalian gelatins were stable longer than those of yellowfin tuna skin gelatin (Fig. 7. B and Fig. 8. B), which indicates that the gel of mammalian gelatins has a higher melting point and more thermostability. The change in viscoelastic properties as a function of time at a constant temperature (20 and 10°C) is presented in Fig. 10. Gelation occurred by physical crosslinking, leading to the formation of junction zones and ultimately a three-dimensional branched network (Gilsenan & Ross-Murphy, 2000). Normand et al. (2000) reported that gelation kinetics could be divided into four phases. The gelatin solution is still liquid in phase 1, and

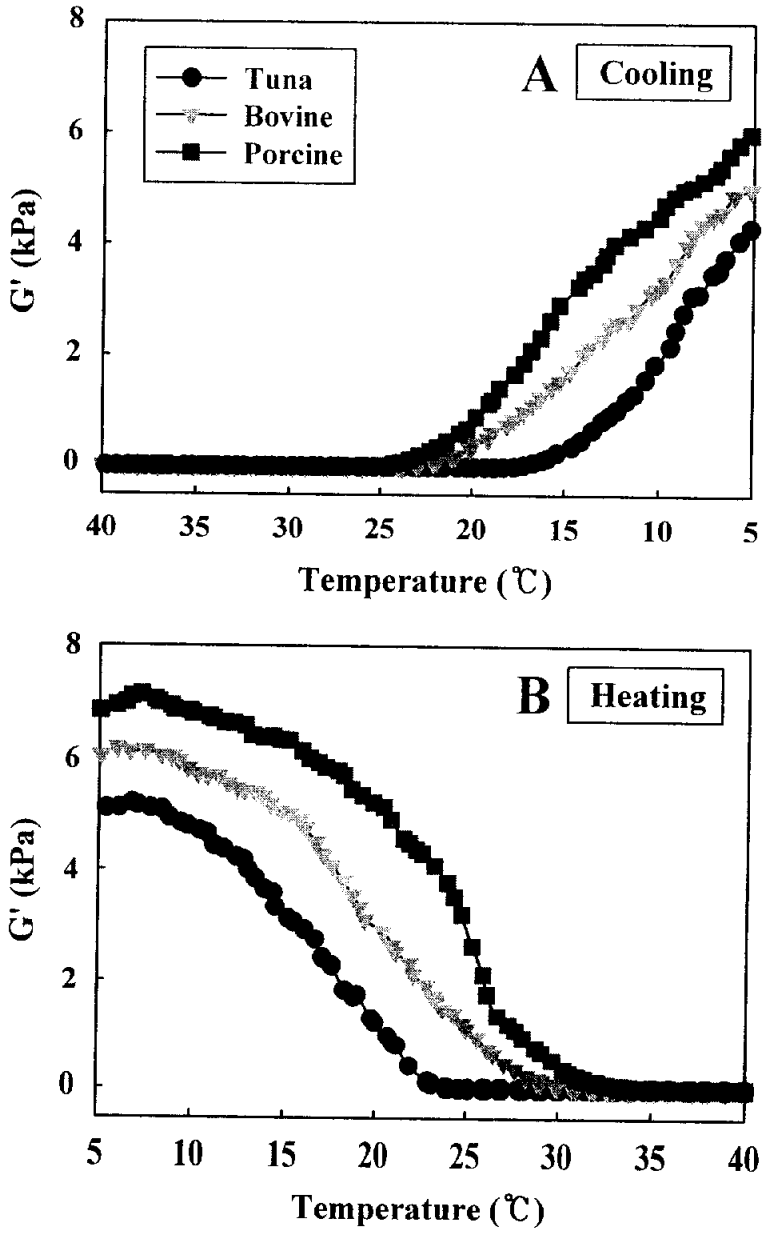

Fig. 7. Evolution of the elastic modulus (G', kPa) during cooling (40°C-5°C) and heating (5°C-40°C) of gelatin solutions. A cooling and heating rate was 0.5°C/min, and a 6.67% (w/v) gelatin solution was used.

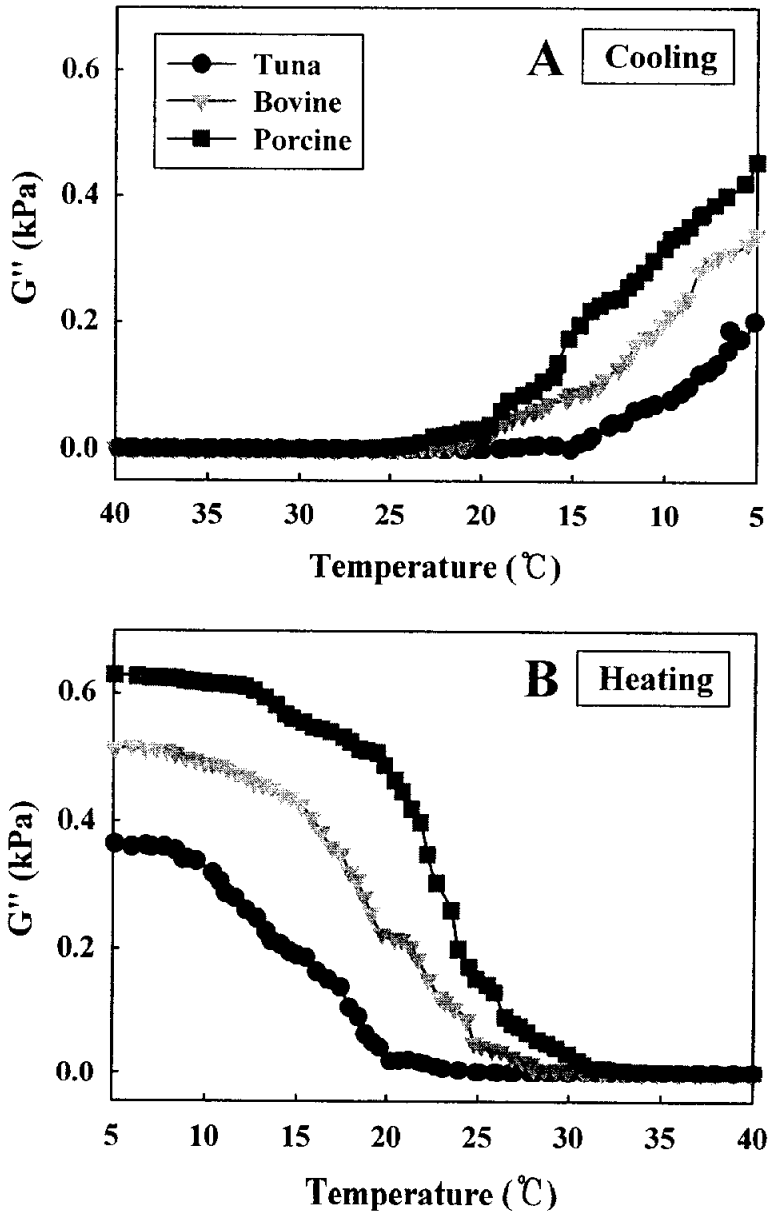

Fig. 8. Evolution of the loss modulus (G", kPa) during cooling (40°C-5°C) and heating (5°C-40°C) of gelatin solutions. A cooling and heating rate was 0.5°C/min, and a 6.67% (w/v) gelatin solution was used.

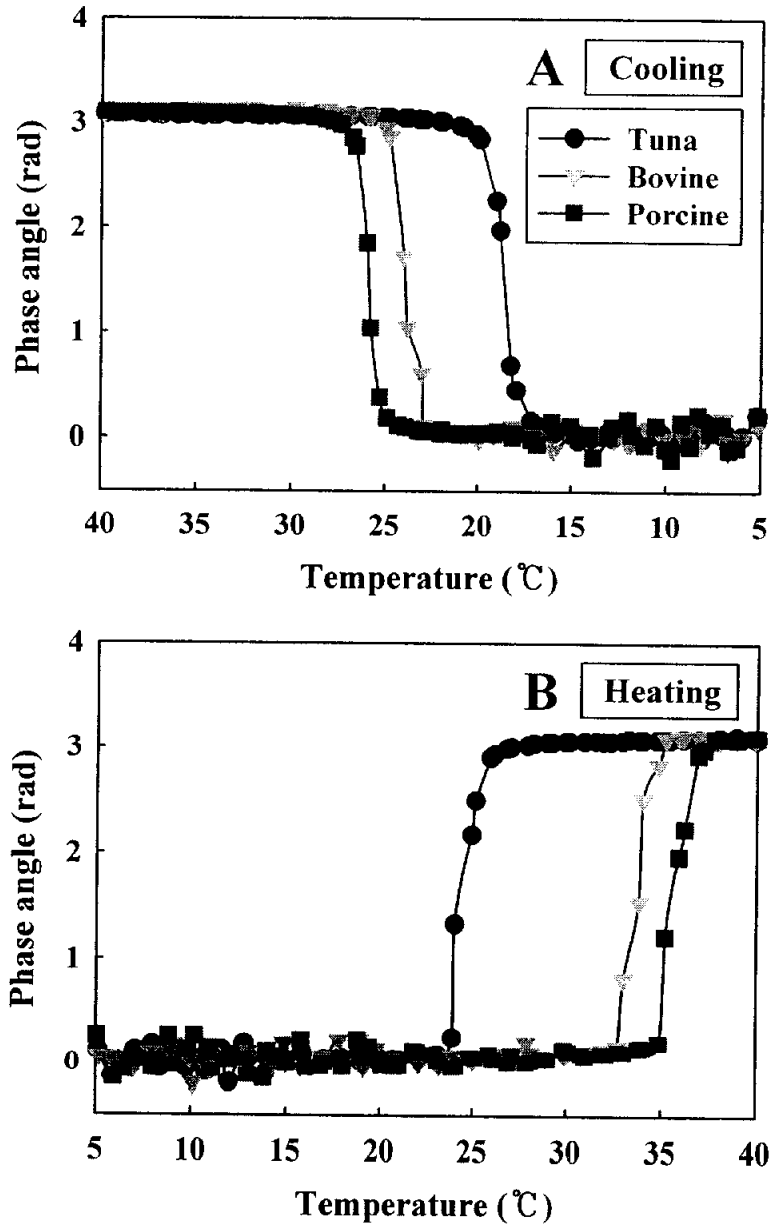

Fig. 9. Evolution of the phase angle (rad) during cooling (40°C-5°C) and heating (5°C-40°C) of gelatin solutions. A cooling and heating rate was 0.5°C/min, and a 6.67% (w/v) gelatin solution was used.

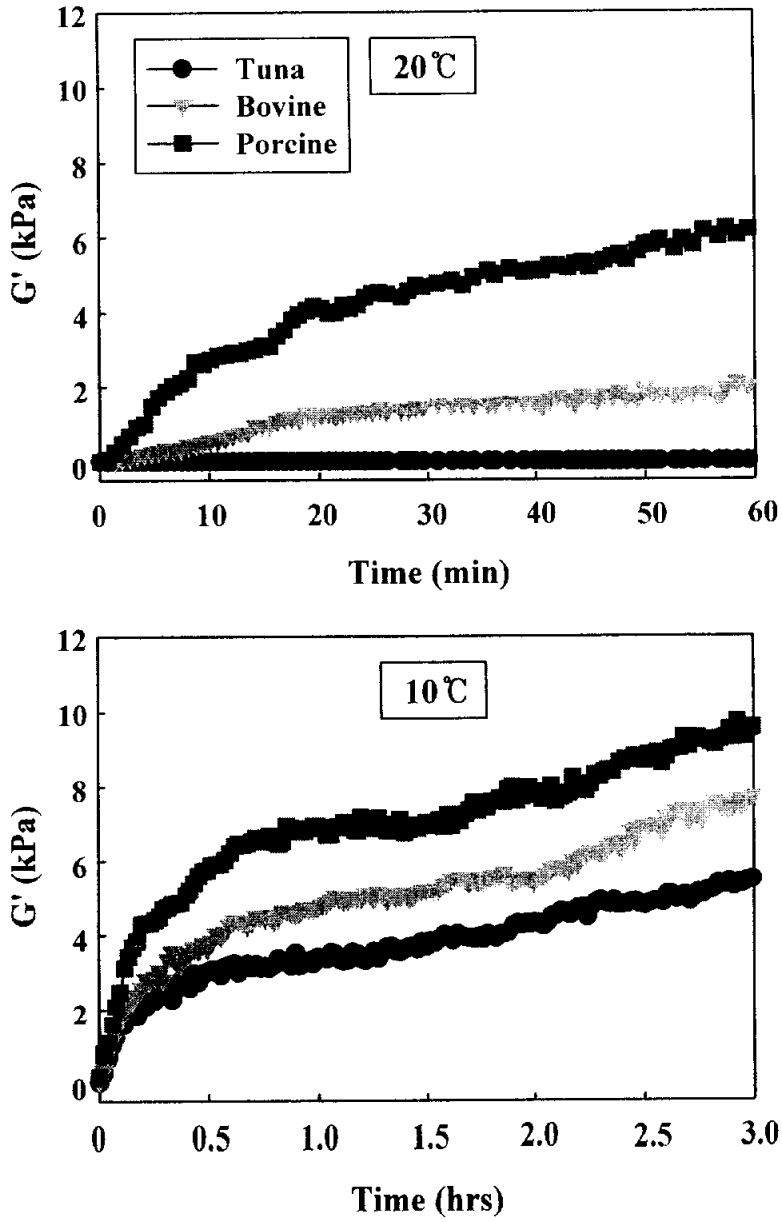

Fig. 10. Evolution of the elastic modulus (G', kPa) as a function of time at constant temperature (20 and 10°C). A 6.67% (w/v) gelatin solution was used.

serious gelation occurs in phase 2 (until 1 hr after phase 1). At phase 2, both G' and G" increase greatly, which indicates rapid formation of junction zones and strong reinforcement of the gel network. During phase 3 (until 100 hrs after phase 2), G' increases more slowly, but G" remains constant. As the result of the elastic modulus (G') at 20°C, bovine and porcine gelatins showed significant increase, while the gelation of yellowfin tuna skin gelatin never occurred for 1 hr (phase 2). In the case of 10°C, yellowfin tuna skin gelatin had a serious increase in G'. The G' increase range of porcine gelatin also was greater than that of bovine gelatin. The G' of all gelatins increased rapidly in the phase 2, and increased slowly after phase 2 as the explanation of Normand et al. (2000). The gelation of yellowfin tuna skin gelatin did not work at room temperature, and required essentially lower temperature below 10°C and longer setting time than mammalian gelatins.

2.4. SDS-PAGE patterns

Collagen, a precursor of gelatin, is comprised of long triple helices containing three interwined α-chains, and in the triple helix molecule, which are held together through hydrogen bonds. There are interstrand (intramolecular) covalent crosslinks in individual molecules, as well as helix-to-helix (intermolecular) covalent crosslinks in collagen fibrils (Nimni, 1998). Ledward (1986) reported that gelatin may retain some helical structure

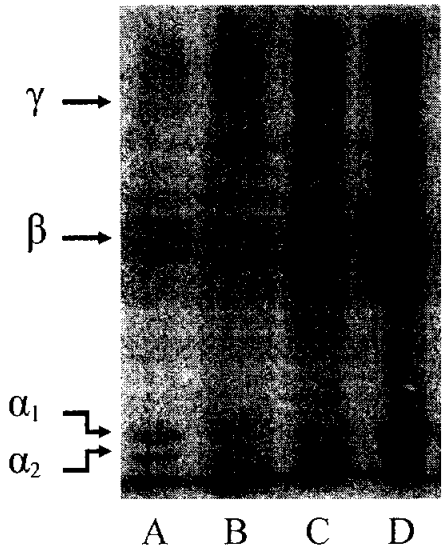

Fig. 11. SDS-PAGE patterns of yellowfin tuna (*T. albacares*), bovine and porcine skin gelatins. 5 mg/mL of gelatin solution, 4% stacking gel and 5% resolving gel were used for electrophoretic analysis. In order to compare molecular weight of sample gelatins, bovine skin type I collagen was used as mobility makers of α-chains, β and γ-components. A, type I collagen from bovine skin; B, bovine skin gelatin; C, porcine skin gelatin; D, yellowfin tuna skin gelatin.

depending on the number of pyrrolidine residues (proline and hydroxyproline) in the gelatin. The gelatins from yellowfin tuna, bovine and porcine were analysed by SDS-PAGE, and type I collagen from bovine skin was used for the comparison of band patterns (Fig. 11). Bovine and fish collagens are essentially composed of α_1 and α_2 chains (a ratio of approximately 2:1), β-component (crosslinked dimer of α-chains) and γ-component (crosslinked trimer of α-chains) (Giraud-Guille et al., 2000). Chang et al. (2000) reported that the molecular weights of α_1, α_2, β and γ collagen subspecies based on the primary sequence were 93, 93, 186 and 279 kDa. Type I collagen showed the band pattern of α_1, α_2, β and γ. The band patterns of α_1, α_2, and β of porcine skin gelatin was similar to that of type I collagen, but bovine skin gelatin had only α-chains. Gelatin from Sigma Chemical Co. has been autoclaved at 121°C for 15-20 min with appreciable hydrolysis in their gelatin processing. Therefore, the absence of β- and γ-components (bovine skin gelatin) and γ-component (porcine skin gelatin) was caused by the degradation of β- and γ-components. Yellowfin tuna skin gelatin presented the band patterns of α_1, α_2, β and γ. The bands of yellowfin tuna skin gelatin were not distinct because a refining process did not added. However, their relative amount was higher than two mammalian gelatins. A greater presence of dimers (β-component) and trimers (γ-component) of α-chains improve better ability of renaturation to the fully collagen native form (Stainsby, 1987). Also. the pure α_1-chain and β-component increase a proportion of

triple helix, and form the strong gel (Sims et al., 1997). Therefore, the gel strength of gelatin from yellowfin tuna skin may be higher than two mammalian gelatins, because of its relatively higher amount of α-chains, β- and γ-component than mammalian gelatins.

2.5. Amino acid composition

Table 12 shows amino acid compositions of yellowfin tuna, bovine and porcine gelatins. The amino acid composition and sequence of gelatin are peculiar in comparison with other proteins, and gelatin always consists of large amounts of glycine (Gly), proline (Pro) and hydroxyproline (Hyp). Gelatin has a repeated structure of Gly-X-Y. When Pro and Hyp were located in the X and Y positions, the thermostability of gelatin was stable. The content of total Gly-Pro-Hyp sequence content is one of the main factors affecting collagen thermostability (Burjandze, 2000). Ledward (1986) and Johnston-Banks (1990) reported that the stability of gelatin is proportional to the total content of pyrrolidine imino acids, which is involved in the formation of junction zones stabilized by hydrogen bonding. The amino acid composition of yellowfin tuna skin gelatin is slightly different from two mammalian gelatins. Fish gelatin tends to have different ratios of amino acids from mammalian gelatin. Because of the different ratio of amino acids, fish gelatin has different functional properties from mammalian gelatin, which is consistent with the results of Arnesen &

Gildberg (2002). The Gly content is typically about 24% for mammalian gelatins and 16-18% for fish gelatins (Gilsenan & Ross-Murphy, 2000), but the content of yellowfin tuna skin gelatin was 24.81%, and surprisingly higher than two mammalian gelatins. The high Gly content of yellowfin tuna skin gelatin may affect the high gel strength. Fish gelatin generally has lower content of imino acids than mammalian gelatins (Ledward, 1986; Norland, 1990; Giraud-Guille et al., 2000). While the imino acid contents (Hyp+Pro) of bovine and porcine gelatins were 25.76% and 26.14%, respectively, that of yellowfin tuna skin gelatin was 24.32%, and lower than two mammalian gelatins as the other fish gelatins. Gilsenan & Ross-Murphy (2000) reported that cold water fish has a very low Hyp content and couples with this a very low gelling and melting temperature; 10% mammalian gelatin forms a gel at room temperature, whereas 10% cod do the gel at 2°C. The gelling and melting points of gelatin has been found to correlate with the proportion of the imino acids, Pro and Hyp (both with a 5-membered pyrrolidine ring) in the original collagen (Veis, 1964). As the results of Cho et al. (2004), the gelling and melting points of yellowfin tuna skin gelatin were lower than bovine and porcine gelatins. Moreover, bovine and porcine gelatins formed gels at 20°C, but yellowfin tuna did not. The present study appears to confirm the role of imino acids as the major determinant for gelling and melting points. Alanine is found in non-polar regions where the sequence of Gly-Pro-Y predominates as the Pro and Hyp (Ledward, 1986; Gómez-Guillén et al., 2002).

Table 12. Amino acid composition of yellowfin tuna (*T. albacares*), bovine and porcine skin gelatins

(%)

Amino acids	Gelatin type		
	Tuna	Bovine	Porcine
Hydroxyproline	9.64	10.29	10.32
Aspartic acid	5.62	5.78	5.64
Threonine	2.30	1.67	1.79
Serine	3.84	3.41	3.34
Glutamic acid	10.62	10.41	10.45
Proline	14.68	15.47	15.82
Glycine	24.81	23.56	23.37
Alanine	9.76	8.92	8.97
Valine	2.26	2.23	2.29
Isoleucine	1.14	1.18	1.14
Leucine	2.75	2.88	2.85
Tyrosine	0.57	0.74	0.79
Phenylalanine	2.31	2.48	2.23
Lysine	3.75	3.52	3.58
Histidine	1.20	1.27	1.24
Arginine	8.09	7.83	7.94
Imino acids[a]	24.32	25.76	26.14

[a] Imino acids mean proline and hydroxyproline.

According to the study of Gómez-Guillén et al. (2002), the gelatin containing the low alanine content has the poor gelling ability. Lysine also stabilizes gelatin structure by forming cross-linking structures between chains. The percentage of alanine and lysine in yellowfin tuna skin gelatin were 9.76 and 3.75, respectively, showing the highest contents. Gel strength is compositely determined by amino acid composition and the amount of α-chains and β-component (Gómez-Guillén et al., 2002). Therefore, the high gel strength of yellowfin tuna skin gelatin can be explained by the high contents of glycine, alanine and lysine, and the high amount of α-chains and β-component.

2.6. The effects of gelatin concentration and maturation time on the gel strength

In order to study the effects of gelatin concentration on the gel strength, the gelatin solutions of yellowfin tuna, bovine and porcine gelatins were prepared with 0.5-6.67% (w/v) concentration. After the gelatin gel was matured at 7°C for 17 hrs, the gel strength was measured. The effect of maturation time was investigated with the gel strength measurement of 6.67% (w/v) gelatin gel, which was formed at 7°C for the different maturation time (from 2 to 16 hrs). Changes in gel strength as the concentration of yellowfin tuna and two mammalian gelatins are shown in Fig. 12. The gel strengths of bovine and porcine gelatins were measured at

1% gelatin concentration due to very weak gels, however yellowfin tuna skin gelatin had the significant gel strength. At gelatin concentration over 1.5%, the gel strength proportionally increased with increasing gelatin concentration up to 6.67%. Regression equations estimated for gelatin concentration are shown in Table 13. The all coefficients of determination (R^2) of equations were higher than 0.95, and were significant statistically because their *P*-value was 0.0001. Ferry (1948) reported that the gel strength was proportional to the square of the concentration of gelatin. This pattern was also observed by Choi & Regenstein (2000). Yellowfin tuna skin gelatin showed the great rate of gel strength, however R^2 of yellowfin tuna skin gelatin was 0.9720, and lower than bovine and porcine gelatins (0.9938 and 0.9930, respectively).

Fig. 13 presents the changes in gel strength of yellowfin tuna and mammalian gelatins with increasing of maturation time. The gel strengths of three gelatins also rose as the increase of maturation time, as well as gelatin concentration. However, from 10 to 16 hrs, there was no significant increase. The gel strengths of two mammalian gelatins showed the inconsiderable increase from about 6 hrs, whereas that of yellowfin tuna skin gelatin rose sharply up to 10 hrs. Particularly, the yellowfin tuna gel matured for 2 hrs had the lower gel strength than porcine gelatin. As the investigation of Cho et al. (2004), the elastic modulus (G') of bovine and porcine gelatins showed more rapid increment than yellowfin tuna skin gelatin. These mean that yellowfin tuna skin gelatin require more maturation

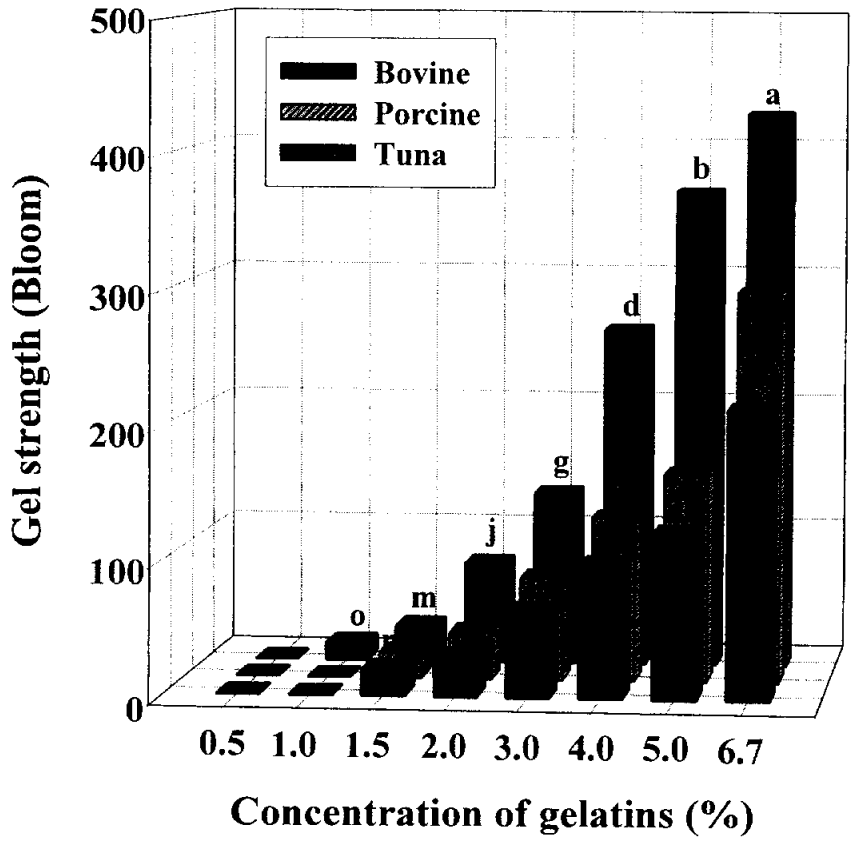

Fig. 12. Changes of the gel strength as affected by the concentration of gelatins. Gelatin solutions were prepared at 60°C, and gelatin gels were matured at 7°C for 17 hrs. Different letters (a, b, c,...) indicate significant differences at an α level of 0.05.

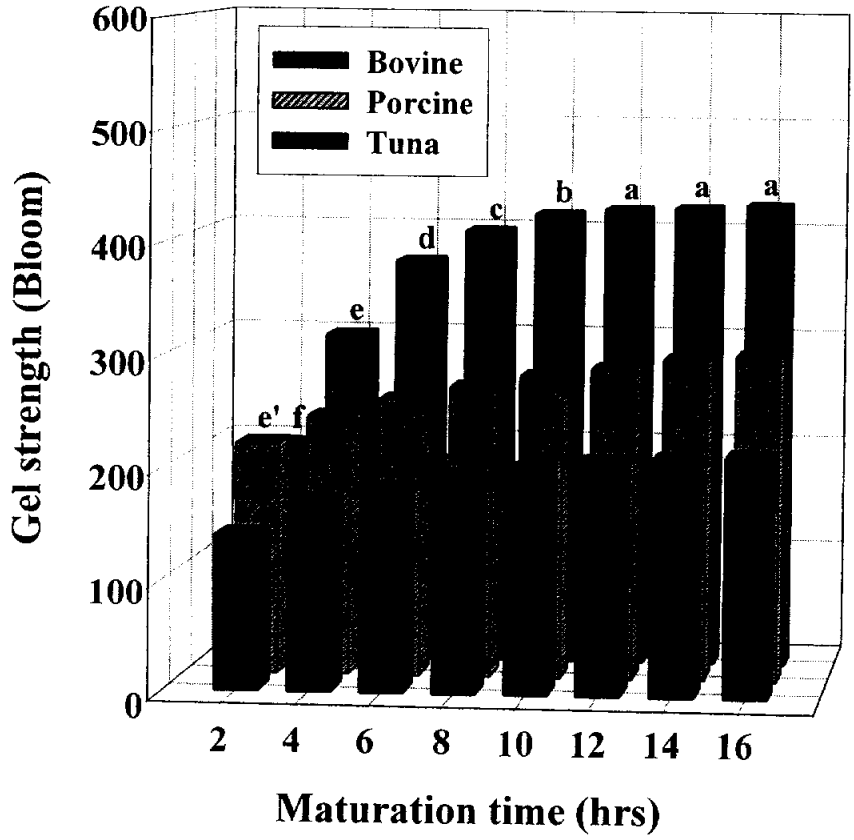

Fig. 13. Changes of the gel strength as affected by the maturation time. Gelatin solutions were prepared with 6.67% (w/v) concentration at 60°C, and gelatin gels were matured at 7°C for 2-16 hrs. Different letters (a, a', a",...) indicate significant differences at an α level of 0.05.

Table 13. Regression equation for the effects of gelatin concentration and maturation time on the gel strength

Gelatin	Regression equation	R^2	P-value
Gelatin concentration			
Y_T (tuna)	$Y_T = -64.52 + 76.26X_1 + 0.09X_1^2$	0.9720	0.0001
Y_B (bovine)	$Y_B = -10.96 + 15.01X_1 + 2.79X_1^2$	0.9938	0.0001
Y_P (porcine)	$Y_P = -9.51 + 11.68X_1 + 4.98X_1^2$	0.9939	0.0001
Maturation time			
Y_T (tuna)	$Y_T = 122.38 + 50.14X_2 - 2.01X_2^2$	0.9712	0.0001
Y_B (bovine)	$Y_B = 121.32 + 12.56X_2 - 0.42X_2^2$	0.9830	0.0001
Y_P (porcine)	$Y_P = 191.25 + 11.85X_2 - 0.34X_2^2$	0.9955	0.0001

Y (gel strength, Bloom), X_1 (gelatin concentration, %), X_2 (maturation time, hrs).

time than mammalian gelatin to form the firm gel. Regression equations of all gelatins for maturation time are significant (all P-value = 0.0001), however the trend of the yellowfin tuna skin gelatin was different from two mammalian gelatins, and also the R^2 value of yellowfin tuna skin gelatin was lower than those of two mammalian gelatins (Table 13).

2.7. The effects of heating and freezing of gelatin solution on the gel strength

Heating and freezing of food ingredients and raw materials are necessary not only to obtain a final food complex but also to preserve the food. Most commercial gelatins are produced through the sterilization process at high temperature, and also bovine and porcine gelatins purchased from Sigma Chemical Co., were autoclaved at 121°C for 15-20 min. Three 6.67% gelatin solutions which were prepared at 60°C were heated at different temperature (70-100°C) for 1-2 hrs. After the heat treatment, gelatin solutions were matured at 7°C for 17 hrs to measure the gel strength. Fig. 14 presents the effects of the heat treatment on the gel strengths of yellowfin tuna skin gelatin and two mammalian gelatins. As the result of the gel strength of gels treated at 70-100°C for 1 hr, yellowfin tuna skin gelatin (21%) decreased sharply in comparison with bovine (12%) and porcine (13%) gelatins, and its thermostability was worse than those of two mammalian gelatins. Two mammalian gelatins showed

the low decrease rate because they had been once heated at 121°C for 15-20 min. This pattern of the gel strength as the heat treatment for 1 hr appears certainly in the case of 2 hrs. The gelatin gels inducted by 2 hrs of heat treatment showed the greater rate of decrease (about 30%) than that of 1 hr heat treatment (about 15%). The gel strength of yellowfin tuna skin gelatin in the case of 1 hr heat treatment was highest, on the other hand, that of the case of 2 hrs was similar with porcine gelatin. Generally, the proteins partially unfold and aggregate at high temperature over their denaturation temperature (Bryant & McClements, 1998). The aggregation of gelatin may inhibit gelatin from forming gel network in the phase of solution. Gŏmez-Guillén et al. (2002) reported that gelatin extracted at 80°C had the very poor gel strength. Cho et al. (2004) represented that shark cartilage gelatin prepared by spray drying also formed the weak gel.

Freezing or freeze drying is common unit operation in the production of protein (Cao et al., 2003), however freezing can induce several stresses that are capable of denaturing proteins, such as cold temperature and ice formation (Franks, 1985). In order to investigate the effect of freezing, 6.67% gelatin solutions were frozen at -20°C for 12 hrs, and then thawed at 60°C. After the maturation of gelatin solutions, the gel strength was measured and this measurement of the gel strength was replicated three times. The effects of freezing of yellowfin tuna, bovine and porcine gelatin solutions on the gel strength are shown in Fig. 15. The gel strengths of all gelatins decreased proportionally as the freezing times. As the investigation

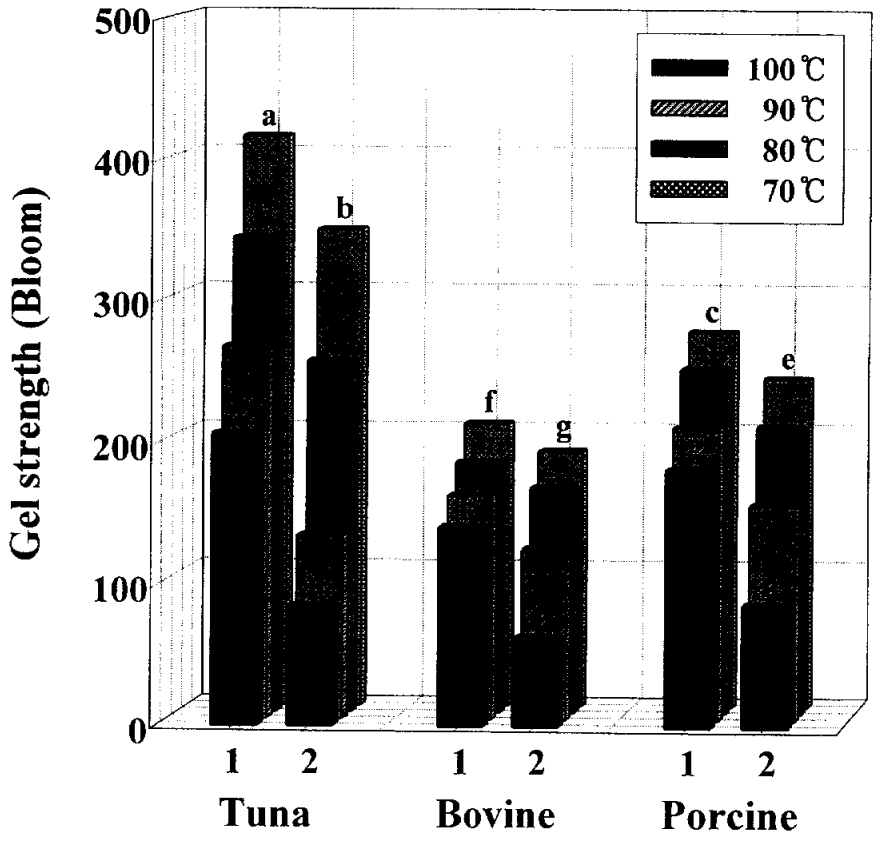

Fig. 14. Changes of the gel strength as affected by the heat treatment of 6.67% (w/v) gelatin solutions. Gelatin solutions were heated at 70-100°C for 1-2 hrs, and then gelatin gels were matured at 7°C for 17 hrs. Different letters (a, b, c,...) indicate significant differences at an α level of 0.05.

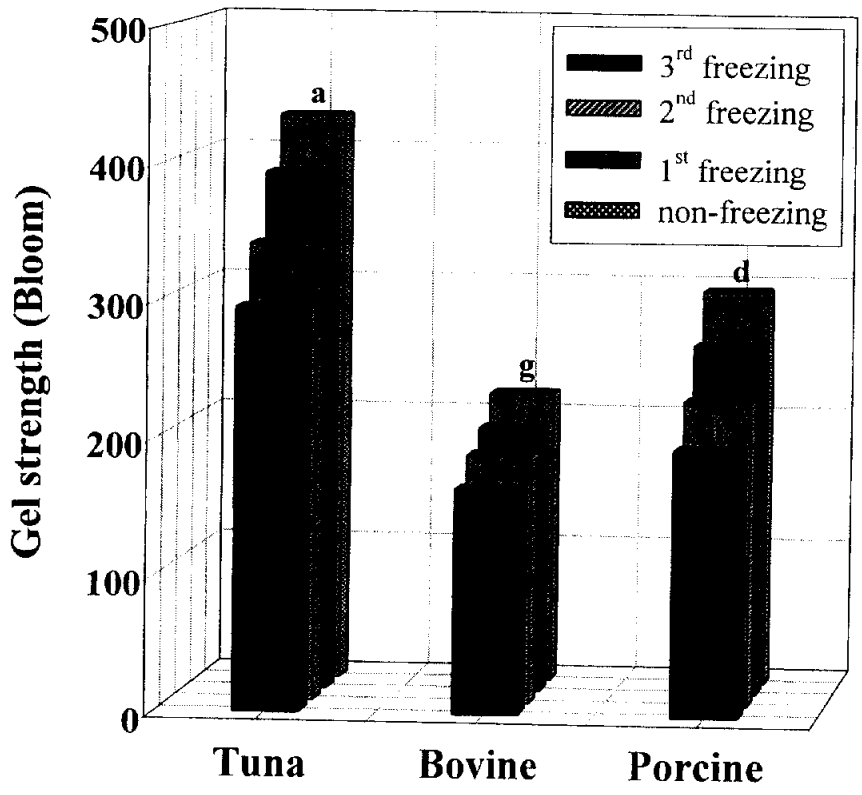

Fig. 15. Changes of the gel strength as affected by the freeze treatment of 6.67% (w/v) gelatin solutions. Gelatin solutions were frozen at −20°C for 10 hrs, and then gelatin gels were matured at 7°C for 17 hrs. Freezing and measurement were repeated 3 times. Different letters (a, b, c,...) indicate significant differences at an α level of 0.05.

of Cepeda et al. (1998), about 10% of protein was denatured during freeze drying. The cold-denaturation of gelatin by freezing resulted in a reduction of the gel strength. In this article, all gelatins showed 8-13% of the decrease rate in the gel strength. Especially, about 13% of the decrease rate of yellowfin tuna and porcine gelatins was higher than about 8% of bovine gelatin. As the result of Fernández-Díaz et al. (2003), the gel strength of gelatin extracted from fresh fish skin is higher than frozen skin gelatins at -12 and -20°C, and cold-denaturation of gelatin derived the decrease of β- and γ-components concerning greatly the formation of gel network. Molecular weight distribution of gelatin is mainly responsible for its gelling behaviour (Johnston-Banks, 1990; Fernández-Díaz et al., 2003). In the present study, bovine gelatin did not show the bands of β- and γ-component. Therefore, it is suggested that the gel strength of yellowfin tuna and porcine gelatins decreased largely because they relatively contain a lot of β- and γ-components.

Conclusion

The gelatin from yellowfin tuna skin showed the limit of gelling and melting temperature in comparison with mammalian gelatins (bovine and porcine). The yellowfin tuna skin gelatin could not form a gel at 20°C, and the elastic modulus (G') and the loss modulus (G") increased at the low temperature of 10°C. Nevertheless, yellowfin tuna gelatin has the highest gel strength, and gelling and melting points are also higher than that of other fish gelatins. The amounts of α-chains, β- and γ-components of yellowfin tuna skin gelatin were higher than those of two mammailan gelatins. Yellowfin tuna skin gelatin showed the lower contents of proline and hydroxyproline. But, the contents of glycine, alanine and lysine of yellowfin tuna skin gelatin were the highest. Yellowfin tuna skin gelatin required more maturation time than two mammalian gelatins to form a firm gel. The skin of yellowfin tuna can be a possible material for gelatin production, and the yellowfin tuna skin gelatin can be used in products requiring very high gel strength. If yellowfin tuna skin gelatin is modified by chemical and enzymatic methods to improve gelling and melting points, it may be offered as the replacement for mammalian gelatins.

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