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Processing Optimization and Physicochemical Characteristics of Collagen from Scales of Yellowfin Tuna (*Thunnus albacares*)

Yuna Han[1], Ju-Ryun Ahn[1], Jin-Wook Woo[2], Cheol-Kyun Jung[1], Sueng-Mock Cho[3], Yang-Bong Lee[1] and Seon-Bong Kim[1]*

[1]Department of Food Science and Technology/Institute of Food Science, Pukyong National University, Busan 608-737, Korea
[2]Food R&D Center, Ourhome Ltd., Sungnam 462-120, Korea
[3]Research Division of Food Industry Promotion, Korea Food Research Institute, Seongnam 463-746, Korea

This study was conducted to investigate the optimal conditions of collagen extraction from scales of yellowfin tuna (*Thunnus albacares*) using surface response methodology. Four independent variables of NaOH concentration and pretreatment fime in alkali pretreatment and enzyme concentration and treatment time in enzyme hydrolysis were used to predict a model equation for the collagen yield. The determinant coefficient (R^2) for the equation was 0.906. The values of the independent variables for the maximum yield were 0.32 N NaOH, 16.38 h alkali pretreatment time, 0.18% enzyme concentration, and 31.02 h enzyme treatment time. In the physicochemical properties of tuna scale collagen, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of tuna scale collagen showed the same migration distances as that of calf skin collagen. The amide A, I, II, and III regions of tuna scale collagen in Fourier transform infrared measurements were shown in the peaks of 3,414 cm^{-1}, 1,645 cm^{-1}, 1,553 cm^{-1}, and 1,247 cm^{-1}, respectively. The amount of imino acids in tuna scale collagen was 18.97% and the collagen denaturation temperature was 33°C. The collagen solubility as a function of NaCl concentration decreased to 4% NaCl (w/v) and the collagen solubility as a function of pH was high at pH 2-4 and sharply decreased from pH 4 to pH 7. Viscosity of the collagen solution decreased continuously until 30°C and this decreasing rate slowed in the temperature range of 35-50°C.

Key words: Collagen, Yellowfin tuna, *Thunnus albacares*, Scale, Response surface methodology, Physicochemical characteristics

Introduction

Collagen is a fibrous protein that composes 25-30% of the total protein in vertebrates. Collagen exists in almost all the tissues and organs of the skin, including cartilage, bone, muscle, blood vessels and supporting tissue, and internal organs to maintain structure (Piez, 1985). Collagen, which has recently been examined as a functional ingredient, has been used as a food additive in ham, sausage, and other foods to improve mouthfeel as well as in film formation, such as edible casings or films (Pearson, 1988). As a medicinal product, type II collagen shows an ability to reduce pain by inhibiting the activity of T cells (Li et al., 2006). Furthermore, collagen can be as an edible condiment due to its efficiency of skin care (Li et al., 2005). However, most collagen research has focused on animal sources of bovine and pork with little research on collagen from marine sources.

Marine sources for collagen have been examined by Nagai and Suzuki (2002). They isolated fish collagen from the epidermis of paper nautilus and the skin of puffer fish, and studied their physicochemical properties, such as molecular weight using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), peptide mapping, and denaturation temperature. Acid-soluble collagens were extracted from the skin of brownstripe red snapper (Jong-

*Corresponding author: owlkim@pknu.ac.kr

jareonrak et al., 2005) and skins of Nile perch (Muyonga et al., 2004). Their properties were studied by thermal denaturation temperature using differential scanning calorimetry, solubility, and amino acid analysis. Yoshimura et al. (2000) and Nomura et al. (2000) extracted collagen from the skin of great blue sharks (*Prionace glauca*) and investigated the physicochemical properties. Also, similar studies were carried out on collagen isolated from the skins of jellyfish and cuttlefish (Nagai et al., 2000, 2001). The collagen from the backbone of *Seriola quinqueradiata* was tested for various uses (Morimura et al., 2002). In the collagen studies of fish scales, collagen was extracted by using 0.5 M acetic acid from carp scales (Kimura et al., 1991). Recently, the partial physicochemical properties of scale collagens from the red sea bream (*Pagrus major*), sardine (*Sardinops melanostictus*), and Japanese sea bass (*Lateolabrax japonicus*) were studied by Nagai et al. (2004). Ikoma et al. (2003) extracted type I collagens from scales of *P. major* and the Nile mouth breeder (*Oreochromis niloticus*) and investigated their properties. Ogawa et al. (2004) isolated collagens from bones and scales of the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*) and examined their biochemical properties. When collagen is isolated from the skin, scale, and other parts of fish, it is used as an ingredient in functional foods, condiments, and medicinal products. Fish collagen can be a viable replacement for the traditional bovine and pork gelatins, which are exposed to bovine spongiform encephalopathy or foot/mouth diseases (Yamauchi, 2002). Also, it can aid in religious dietary laws stating that the bovine or pork collagens cannot be used (Sadowska et al., 2003). Furthermore, collagen extraction from fish by-products reduces environmental contamination and produces biofunctional proteins.

Tuna (skipjack and yellowfin) have been a staple fish and their harvesting amount is estimated at 4,060,000 MT per year (2007 World Capture Production of FAO Fisheries Department). Yellowfin tuna is mainly consumed as sashimi in sushi and canned foods, and most skipjack tuna is used for canned foods and hatsuobushi. However, many of the processing by-products such as skin, scale, and others induce environmental pollution. Therefore, the extraction of bioactive components from the by-products is an efficient and economically advantageous means of recycling these waste products.

Response surface methodology (RSM), which shows the relationship between dependent variables and independent variables, has been used to determine the optimum extraction conditions for bioactive components (Edwards and Jutan, 1997). Using RSM, Cho et al. (2004, 2005) studied the optimization of gelatin extraction from shark (*Isurus oxyrinchus*) cartilage and yellowfin tuna skin. These studies demonstrated that RSM is an effective tool for investigating the optimum extraction conditions for producing gelatin.

The objectives of this research were to investigate the optimum conditions for extracting collagen from the scales of yellowfin tuna using RSM and to investigate the physicochemical properties of the resulting collagen. Its physicochemical properties are also compared with those of collagen from calf skin.

Materials and Methods

Materials

Yellowfin tuna (*Thunnus albacares*) scale was obtained from Dongwon Industries Co., Ltd. (Busan, Korea, 2006) and stored at -18°C until use. Calf skin collagen and pepsin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The other chemicals used for this study were of analytical grade.

Methods

Collagen extraction

Collagen extraction was carried out by the method of Ogawa et al. (2004), with slight modification. Yellowfin tuna scale was homogenized for 3 min at 10,000×*g* (HB-201SF; Hanbaek Scientific Co., Seoul, Korea) with a homogenizer (CR-52F; SMT Co., Tokyo, Japan). The homogenized scale was washed and added into 0.1, 0.2, 0.3, 0.4, and 0.5 M NaOH to remove non-collagen proteins prior to shaking at 4°C for 6 to 30 h with 6 h intervals in a shaking incubator (NB-205v; N-BIOTEK, Tokyo, Japan). After alkali pretreatment, the alkali solution was removed via washing and neutralization. The neutralized sample solution was diluted to 10 times (w/v) with 0.04, 0.12, 0.20, 0.28, and 0.36% acetic acid solution, which was added with pepsin (0.04-0.36%, w/v). Enzymatic hydrolysis was carried out at 10°C for 18 to 42 h with 6 h intervals in a shaking incubator at 200 rpm. Enzyme-treated samples were separated into extracted solution and residue using filter paper (5A 110 mm; Advantec, Tokyo, Japan). Extracted solution was dialyzed with 25% NaCl solution to make a 5% final concentration. Dialyzed solution was diluted with 10 times distilled water and centrifuged three or four

Table 1. Experimental ranges and values of the independent variables in the central composite design for collagen processing from scale of yellowfin tuna (*Thunnus albacares*)

Independent variables	Symbol	Ranges and levels				
		-2	-1	0	1	2
NaOH concentration (N)	X_1	0.1	0.2	0.3	0.4	0.5
NaOH pretreatment time (hr)	X_2	6	12	18	24	30
Enzyme concentration (%)	X_3	0.04	0.12	0.2	0.28	0.36
Hydrolysis time (hr)	X_4	18	24	30	36	42

times at 10,000×*g* before drying. The whole procedure for collagen extraction was carried out at low temperatures to prevent heat denaturation of collagen.

Experimental design and data analysis

To determine the optimum extraction conditions, central composite design (CCD) (Box & Wilson, 1951) was attempted in the experimental range (Table 1). Regression analysis on the response surface was carried out using SAS (version 9.01; SAS Institute, Cary, NC, USA). Four independent variables were set up and each variable was designed in the range of five values. Twenty-seven experimental points composed of 16 factorial points, 8 axial points, and 3 central points (Table 2) were designed. The important variables for collagen extraction were chosen based on the four independent variables of concentration and time for alkali and enzyme

treatments. That is, the independent variables were NaOH concentration (N, X_1), alkali pretreatment time (h, X_2), pepsin concentration (%, X_3), and pepsin treatment time (h, X_4). The ranges and levels were decided with five intervals based on the formula (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where x_i is the coded value of the independent variable, X_i is the real value of the independent variable, X_0 is the real central value of the independent variable, and ΔX_i is the range of the independent variable. The dependent variable was collagen yield (Y, %) (Table 2). The central points and the ranges for the independent variables were set based on pre-experimental results (Table 1). The response surface model equation was obtained at the 95% confidence level using the RSREG (response surface regression) procedure of SAS version 9.01:

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

where Y is the collagen yield (%) of the dependent variable; β_0 is a constant; β_i, β_{ii}, and β_{ij} are regression coefficients; and X_i and X_j are independent values. The response surface plots of the three-dimensional graph were drawn using Maple 7 (Waterloo Maple Inc., Waterloo, ON, Canada). When two independent variables were shown for collagen yield, the other two independent variables were fixed to the values under the optimum condition.

Table 2. Central composite design and responses of the dependent variable for collagen processing from scale of yellowfin tuna (*Thunnus albacares*) to the independent variables

Run No.	Coded levels of variable				Response
	X_1	X_2	X_3	X_4	Y (%)
1	-1	-1	-1	-1	1.84
2	-1	-1	-1	+1	4.82
3	-1	-1	+1	-1	3.93
4	-1	-1	+1	+1	2.19
5	-1	+1	-1	-1	1.77
6	-1	+1	-1	+1	2.24
7	-1	+1	+1	-1	2.16
8	-1	+1	+1	+1	1.88
9	+1	-1	-1	-1	3.53
10	+1	-1	-1	+1	3.93
11	+1	-1	+1	-1	3.53
12	+1	-1	+1	+1	3.34
13	+1	+1	-1	-1	2.04
14	+1	+1	-1	+1	2.79
15	+1	+1	+1	-1	1.57
16	+1	+1	+1	+1	2.36
17	-2	0	0	0	2.32
18	+2	0	0	0	2.36
19	0	-2	0	0	3.06
20	0	+2	0	0	1.37
21	0	0	-2	0	2.55
22	0	0	+2	0	1.45
23	0	0	0	-2	1.88
24	0	0	0	+2	3.57
25	0	0	0	0	6.95
26	0	0	0	0	6.28
27	0	0	0	0	6.59

SDS-PAGE

SDS-PAGE was carried using a Mini-Protean 3 (Bio-Rad Laboratories, Hercules, CA, USA). The condition of the gel electrophoresis was slightly modified from the method of Laemmli (1970). For polyacrylamide gel, a 5% concentration of stacking gel and resolving gel were used. The 5 mg/mL of sample was equally mixed with the buffer, which contained 1 mL of 0.25 M Tris-HCl (pH 6.8), 1.6 mL

of 10% SDS, 0.4 mL of 5% 2-mercaptoethanol, 3.2 mL of 20% glycerol, and 1 mL of 0.1% bromophenol blue. The mixed solution was heated at 100°C for 3 min. The 5 uL of sample solution was injected to the gel, and then 20 mA gel current was flowed at ambient temperature for 2 h. After gel electrophoresis, the gel was stained with 0.25% (w/v) Coomassie brilliant blue R250. Calf skin was used as a marker protein for comparison.

Amino acid analysis

A 5 mg collagen sample was weighed and 3 mL of 6 N HCl was added before hydrolyzing at 110°C for 24 h in a dry bath. The hydrolyzed solution was vacuum-filtered with a glass filter (0.5 μm) and vacuum-concentrated using a rotary vacuum evaporator (N-1N8; EYELA, Tokyo, Japan). The concentrated solution was made to 10 mL final volume with a sodium citrate phosphate buffer (pH 2.2). The amino acid analysis was carried out using an automatic amino acid analyzer (L-8800; Hitachi, Tokyo, Japan).

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy was measured using a FT-IR spectrophotometer (IFS 88; Bruker, Ettlingen, Germany). Data were collected from 500 to 4,000 cm^{-1} with a 2 cm^{-1} data acquisition rate for collagens from yellowfin tuna scale and calf skin. Curve fitting was shown using Peakfit Software (SPSS Inc., Chicago, IL, USA).

Viscosity measurement

The viscosity of collagen from yellowfin tuna scale was measured using a slightly modified method of Kittiphattanabawon et al. (2005). Collagen was dissolved in 0.1 M acetic acid solution to make a 500 mL final volume of a 0.03% collagen concentration. The solution was melted in a 60°C water bath (SB-651; EYELA). A Brookfield Synchorolectic viscometer (Model II+; Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) was used at 30 rpm with a 40 spindle number. The temperature was measured from 15°C to 50°C, increasing at 5°C/min. The collagen solution was kept for 10 min at each temperature and the experiment was performed with three replications. The viscosity at each temperature was compared with the result at 15°C.

Solubility measurement

Collagen solubility was measured using the method of Montero et al. (1991), with a slight modification. The final collagen concentrations were made to 3 and 6 mg/mL with 0.5 M acetic acid solution, which was stirred at 4°C for 12 h to melt it. Collagen solubility at each NaCl concentration was measured. Each 5 mL NaCl solution, which contained 0%, 2%, 4%, 6%, 8%, 10%, or 12% (w/v) NaCl in a 0.5 M acetic acid solution, was mixed to a 5 mL collagen solution with a 6 mg/mL collagen concentration. Therefore, the final concentrations of each solution were 0%, 1%, 2%, 3%, 4%, 5%, and 6%, respectively. The above solution was stirred at 4°C for 30 min and centrifuged at 4°C and 10,000×*g* for 30 min. The protein concentration of the supernatant was measured by the method of Lowry et al. (1951) and bovine serum albumin was used as a standard. The relative solubilities of NaCl concentrations are shown in comparison with the value of the highest solubility. The effect of pH on collagen solubility was measured. The pH of an 8 mL collagen solution of 3 mg/mL concentration was adjusted from 1 to 10 with 6 N NaOH or 6 N HCl. The final volume was adjusted to 10 mL with distilled water. The solution was centrifuged at 4°C and 10,000×*g* for 30 min. The protein concentrations were determined as described previously.

Measurement of collagen denaturation temperature

Collagen denaturation temperature, calculated using a modified method of Kimura et al. (1988), was determined by measuring its viscosity. After making 5 mL of 0.03% collagen concentration, the collagen viscosity was measured using an Ostwald-Fenske viscometer. Viscosity of the collagen solution was measured from 20°C to 50°C with 3°C intervals. Each solution temperature was kept for 10 min in a water bath (SB-651; EYELA). Collagen denaturation temperature (T_d) was determined to be the temperature at 50% of the initial measured value.

Results and Discussion

Statistical analysis on collagen extraction

To optimize collagen processing from scales of yellowfin tuna, 27 experimental points were set. The results reported as collagen yield (Y, %) are shown in Table 2. From the data, the second-order model equation was obtained using the RSREG procedure of SAS software. The significance tests on all coefficients for the first-order terms (X_1, X_2, X_3, and X_4), the second-order terms (X_{11}, X_{22}, X_{33}, and X_{44}), and cross-products were evaluated using *t*-statistics, and their results are shown in Table 3.

Table 3. Estimated coefficients of the fitted quadratic polynomial equation for the response of Y (yield, %) based on t-statistic

Parameter[a]	Parameter estimate	Standard error	T-value	P-value
Intercept	6.6067	0.4036	16.37	<.0001
X_1	0.0975	0.1427	0.68	0.5074
X_2	-0.5700	0.1427	-3.99	0.0018
X_3	-0.1750	0.1427	-1.23	0.2435
X_4	0.2733	0.1427	1.92	0.0795
X_1X_1	-0.9958	0.1513	-6.58	<.0001
X_1X_2	-0.0525	0.1748	-0.30	0.7690
X_2X_2	-0.0271	0.1513	-6.79	<.0001
X_1X_3	-0.0613	0.1748	-0.35	0.7320
X_3X_2	0.0163	0.1748	0.09	0.9274
X_3X_3	-0.0808	0.1513	-7.14	<.0001
X_4X_1	0.0200	0.1747	0.11	0.9108
X_4X_2	0.0175	0.1747	0.10	0.9219
X_3X_4	-0.3763	0.1747	-2.15	0.0524
X_4X_4	-0.8996	0.1513	-5.94	<.0001

[a]X_1, X_2, X_3, and X_4 mean NaOH concentration (N), alkali pretreatment time (hr), enzyme concentration (%), and enzyme treatment time (hr), respectively.

While X_1 (P=0.5074), X_3 (P=0.2435), and X_4 (P=0.0795) were not significant (P>0.05), X_2 (P=0.0018) was significant (P<0.05). While all the second-order terms were significant (P<0.01), all cross-products were not (P>0.05).

The reaction model equation of the surface response method obtained after eight terms that were not significant at 95% were removed (Table 4).

The determinant coefficient (R^2) on collagen yield was 0.906 (P=0.0004). The reason for the high determinant coefficient and significance was probably due to the central points and ranges that were chosen by pre-experimental data. The second-order model equation for collagen yield (Y, %) was evaluated using analysis of variance (ANOVA) (Table 5). In the ANOVA results, the first-order terms (X_1, X_2, X_3, and X_4; P=0.0101) and the second-order terms (X_{11}, X_{22}, X_{33}, and X_{44}; P<0.0001) were significant at a significance level of 95%, except for cross-product terms (P=0.58). The regression was significant at P<0.01 and the model equation for the dependent variable (Y, %) was shown to be significant at a 95% significance level due to the lack of fit (Table 4).

Table 4. Analysis of variance (ANOVA) for response of the dependent variable (Y, yield(%))

Sources	DF[a]	SS[a]	MS[a]	F-value	P-value
Regression					
Linear	4	10.55	0.17	5.40	0.0101
Quadratic	4	43.29	0.70	22.15	<.0001
Cross-product	6	2.38	0.04	0.81	0.5795
Model	14	56.23	0.91	8.22	0.0004
Residual					
Lack of fin	10	5.64	0.56	5.01	0.18
Pure error	2	0.22	0.11	-	-
Total error	12	5.86	0.49	-	-
Total	26	62.09		-	-
Factors[b]					
X_1	5	21.49	4.30	8.80	0.0010
X_2	5	30.36	6.07	12.43	0.0002
X_3	5	27.99	5.60	11.46	0.0003
X_4	5	21.33	4.27	8.73	0.0011

[a]DF, SS and MS stand for degrees of freedom, sum of square and mean square, respectively.
[b]X_1, X_2, X_3, and X_4 mean NaOH concentration (N), alkali pretreatment time (hr), enzyme concentration (%), and enzyme treatment time (hr), respectively.

Table 5. Optimal conditions of collagen processing from scale of yellowfin tuna (*Thunnus albacares*)

Dependent variable	Independent variables[a]	Critical value		Predicted yield (%)	Stationary point
		Coded	Uncoded		
Y (Yield, %)	X_1	0.06	00.32	6.72%	Maximum
	X_2	-0.27	16.38		
	X_3	-0.11	00.18		
	X_4	0.17	31.02		

[a]X_1, X_2, X_3, and X_4 mean NaOH concentration (N), alkali treatment time (hr), enzyme concentration (%), and enzyme pretreatment time (hr), respectively.

Optimization of collagen extraction

CCD (Box & Wilson, 1951) was used to investigate the optimum condition of collagen extraction from scales of yellowfin tuna. The four independent variables were NaOH concentration (0.3 N, X_1) and pretreatment time (18 h, X_2), and pepsin concentration (0.2%, X_3) and treatment time (30 h, X_4,). Their central points and the ranges were determined by pre-experimental results (Table 1). From the RSM results, the coded and uncoded values at the optimum levels are shown in Table 5.

Based on the results of the RSREG procedure, all the eigenvalues are negative, showing that the equation has a maximum value. The coded values at the optimum levels for maximum collagen extraction from yellowfin tuna scale were x_1=0.06, x_2=-0.27, x_3=-0.11, and x_4=0.17. That is, the optimum condition for maximum collagen extraction was 0.32 N NaOH concentration (X_1), 16.38 h alkali pretreatment time (X_2), 0.18% pepsin concentration (X_3), and 31.02 h pepsin treatment time (X_4) (Fig. 1).

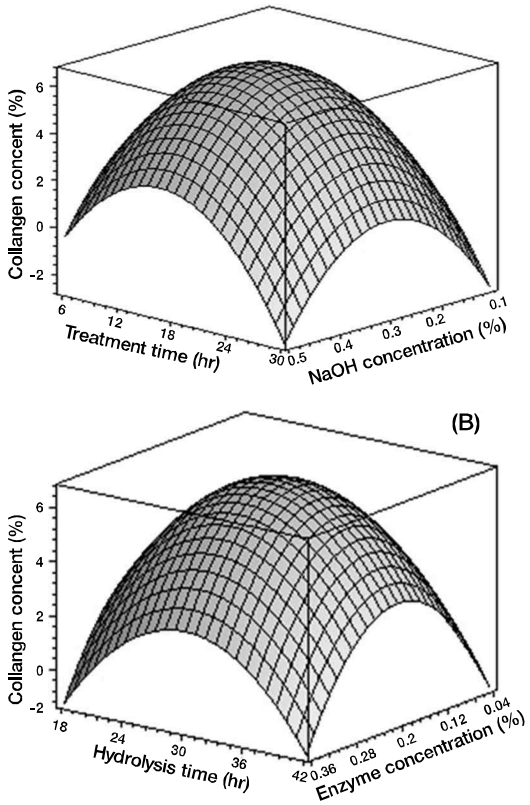

Fig. 1. Response surface plots for optimization of collagen extraction from scale of Yellowfin tuna scale. Y, X_1, X_2, X_3, and X_4 stand for collagen content (%), NaOH concentration (N), alkali pretreatment time (hr), enzyme concentration (%), and hydrolysis time (hr), respectively.

Under the above conditions, the expected collagen yield (Y, yield (%)) was 6.72% and the experimental collagen yield was 6.93%. The extracted collagen yield was similar to the expected collagen yield.

The effects of the independent variables (X_1, X_2, X_3, and X_4) on collagen yield (Y) were drawn as three-dimensional graphs using Maple 7 (Fig. 1). The relationship between alkali concentration and pretreatment time on collagen yield is shown in Fig. 1a and the relationship between pepsin concentration and treatment time on collagen yield is given in Fig. 1b. Both graphs show that the collagen yield increased as coded values were close to zero.

SDS-PAGE of collagen

The patterns of SDS-PAGE for yellowfin tuna collagen and calf skin collagen are shown in Fig. 2.

Calf skin collagen is composed of an α_1-chain and an α_2-chain. Type I collagen consists of two identical α_1- and α_2-chains (Wong, 1989; Burghagen, 1999) and a β-component (cross-linked dimer of α-chains) and γ-component (cross-linked trimer of α-chains). Giraud-Guille et al. (2000) reported that the molecular weights of α_1, α_2, β, and γ were 93, 93, 186, and 279 kDa, respectively. When compared to calf skin

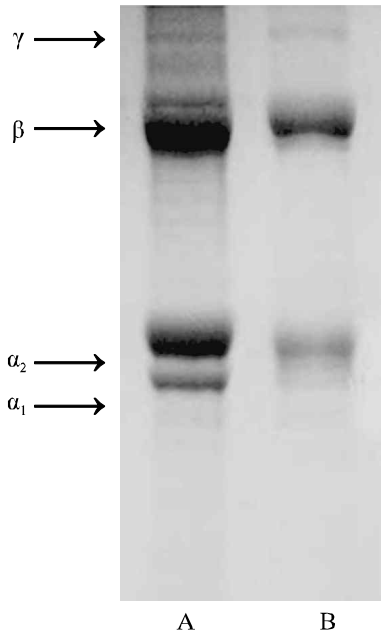

Fig. 2. SDS-PAGE patterns of collagen from scale of yellowfin tuna (*Thunnus albacares*) and calf skin. A; collagen from calf skin, B; collagen from scales of yellowfin tuna. The 5% separating gel and 4% stacking gel were used for eletrophoretic analysis. Loading volume of sample solution is 5.0 mg/mL. Calf skin collagen was used as mobility maker of α, β and γ-chain components.

collagen, yellowfin tuna scale collagen had an α_1-chain, an α_2-chain, a β-chain, and a γ-chain, and their α_1- and α_2-chains were less dense.

Amino acid composition of collagen

The amino acid composition of collagen from yellowfin tuna scale and calf skin collagen is shown in Table 6.

Table 6. Amino acid composition of collagens from yellowfin tuna (*Thunnus albacares*) scale and calf skin (%)

Amino acids	Collagen type	
	Calf skin	Yellowfin tuna scale
Hydroxyproline	9.06	7.43
Aspartic acid	5.11	6.83
Threonine	1.85	1.40
Serine	2.85	2.45
Glutamic acid	10.69	11.20
Proline	10.88	11.54
Glycine	16.28	14.44
Alanine	8.12	8.65
Valine	2.27	2.25
Isoleucine	1.37	1.84
Leucine	2.86	3.50
Tyrosine	0.48	0.17
Phenylalanine	1.84	1.59
Lysine	0.27	0.53
Histidine	1.96	2.31
Arginine	4.19	4.91
Cysteine	0.01	-
Imino acids[a]	19.93	18.97

[a]Imino acids mean proline and hydroxyproline.

The amount of hydroxyproline residue for collagen from yellowfin tuna scales was 7.43%, which was lower than that of calf skin collagen (9.06%). However, the amount of proline residue for collagen from yellowfin tuna scales was 11.54%, which was higher than that of calf skin collagen (10.88%). Glycine was the most prevalent amino acid in both calf and yellowfin tuna scale collagens at 16.28% and 14.44%, respectively. The amount of hydroxyproline has been known to affect rheological properties, including gel strength. Gel produced from collagen peptide was reported to have better quality with a higher amount of hydroxyproline (Gilsenan & Ross-Murphy, 2000; Gómez-Guillén et al., 2002). Therefore, the residual amounts of proline and hydroxyproline are thought to be important for collagen peptide quality.

FT-IR

FT-IR spectra for collagens from yellowfin tuna scales and for calf skin are shown in Fig. 3.

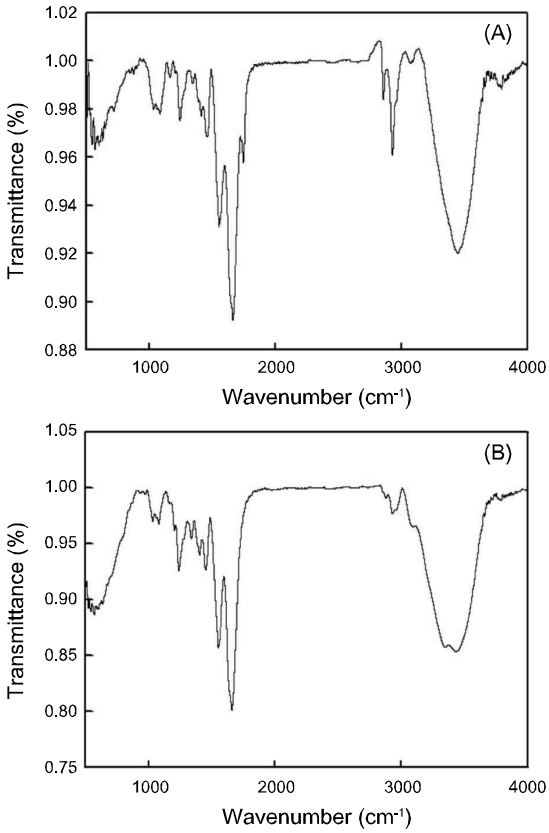

Fig. 3. Fourier transform infrared spectra of collagen from yellowfin tuna scale (A) and calf skin (B).

The regions of amide A, I, II, and III in the FT-IR spectrum are known to be associated with the structure of polypeptides (Jackson et al., 1995). The amide A band (3,400-3,440 cm^{-1}) is known to associate with N-H stretching vibration. The amide III band (1,600-1,660 cm^{-1}) is related to stretching vibrations of the carbonyl group in peptides, and it is used to determine the secondary structure of proteins. Amide A (1,550 cm^{-1}) is related to N-H bending and C-N stretching. Amide III (1,320-1,220 cm^{-1}) is shown in C-N stretching and N-H stretching in collagen triple helical conformations (Jakobsen et al., 1983; Muyonga et al., 2004). In this study, the respective regions of amide A, I, II, and III for collagen from scales of yellowfin tuna and calf skin collagen were 3,435 cm^{-1} and 3,414 cm^{-1}, 1,652 cm^{-1} and 1,645 cm^{-1}, 1,554 cm^{-1} and 1,553 cm^{-1}, and 1,240 cm^{-1} and 1,247 cm^{-1}, showing the similar peak wavelengths. In the results of Muyonga et al. (2004), collagen from the skin of young Nile perch showed 3,434, 1,650, 1,542 and 1,235 cm^{-1} for the A, I, II, and III amide regions, respectively. Collagen from the adult skin of Nile perch had peaks at 3,458, 1,654,

1,555, and 1,238 cm^{-1} for the A, I, II, and III amide regions, respectively

Collagen viscosity

A concentration of 0.04% (w/v) collagen was dissolved in distilled water at 60°C. The viscosity of 0.04% (w/v) collagen solution from scales of yellowfin tuna constantly decreased until 32°C and this decreasing rate slowed from 35°C to 50°C (Fig. 4).

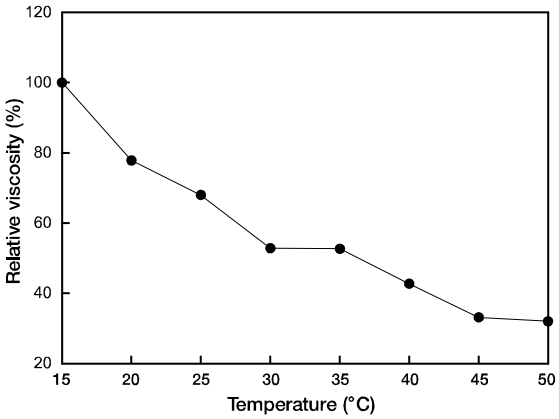

Fig. 4. Relative viscosity at different temperatures of the collagen solution of scale of yellowfin tuna (*Thunnus albacares*).

A high-temperature treatment induces the breakage of hydrogen bonding in gelatin structures (Wong, 1989). Changes in the gelatin structure during heating affects its viscosity (Nagai et al., 1999; Nagai & Suzuki, 2000, 2002). Kittiphattanabawon et al. (2005) reported that the viscosities for collagens of bones and skins of bigeye snapper decreased constantly until 30°C and this decreasing rate was reduced in the temperature range of 35-50°C. Also, the changes in gelatin viscosity for jellyfish showed a similar tendency as those of collagens of the bones and skins of bigeye snappers.

Collagen solubility

The effects of NaCl concentration on collagen solubility are shown in Fig. 5.

Solubility of collagen from scales of yellowfin tuna slowly decreased until 4% (w/v) NaCl. After that, its solubility slowly increased with NaCl concentration. An increase in ionic strength causes a reduction in protein solubility via an enhanced hydrophobic-hydrophobic interaction between protein chains and the competition for water of ionic salts, leading to the induced protein precipitation (Vojdani, 1996; Jongjareonrak et al., 2005).

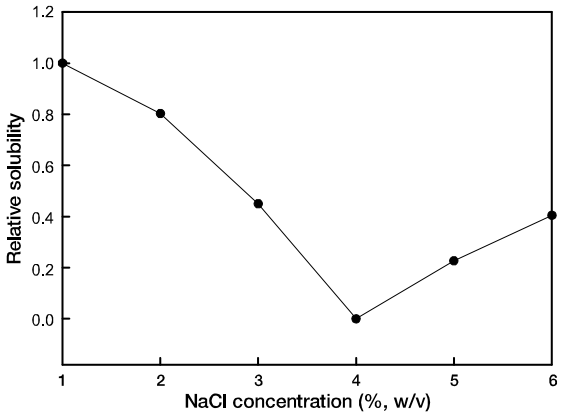

Fig. 5. Solubility in 0.5 M acetic acid on NaCl concentration of the collagen obtained from scale of yellowfin tuna (*Thunnus albacares*).

In studies on collagen solubility in several NaCl concentrations on other fish, Kittiphattanabawon et al. (2005) reported that acid-soluble collagens from the skins and bones of the bigeye snapper showed almost the same solubility in 0-3% NaCl concentration. In a high NaCl concentration, its decreased solubility is thought to be due to salting out. That is, a higher NaCl concentration in a protein solution induces its lower solubility.

The effects of pH on the solubility of collagen from the scales of yellowfin tuna are shown in Fig. 6.

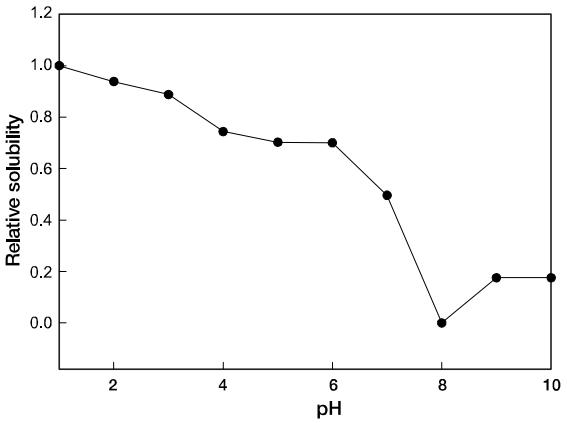

Fig. 6. Solubility in 0.5M acetic acid on pH of the collagen solution from scale of yellowfin tuna (*Thunnus albacares*).

Collagen solubility was highest at pH 1 and it slowly decreased in the range of pH 2-6. Then, it sharply decreased in the range of pH 6-8. Collagen solubility slowly increased in the range of pH 9-10. In general, collagen shows high solubility under acidic conditions and the different solubilities of

proteins at different pH levels are thought to be due to their different isoelectric points (pIs). When the pH of a protein solution is higher or lower than its pI, protein solubility increases due to the increased repulsion of protein residuals. In the opposite situation at the pI, hydrophobic interactions increase and the total charge of a protein molecule is close to zero; so protein aggregation is induced (Wong, 1989).

The solubilities of collagens from bigeye snapper bone and skin have been reported to be highest at pH 2 and 5, respectively. Their solubilities sharply decreased after passing pH 5 and pH 6, and their solubilities were lowest at pH 7 and 8, respectively. The solubilities were similar to those reported in this study (Kittiphattanabawon et al., 2005).

Collagen denaturation temperature (T_d)

The denaturation temperature (T_d) of yellowfin tuna scale collagen is shown in Fig. 7.

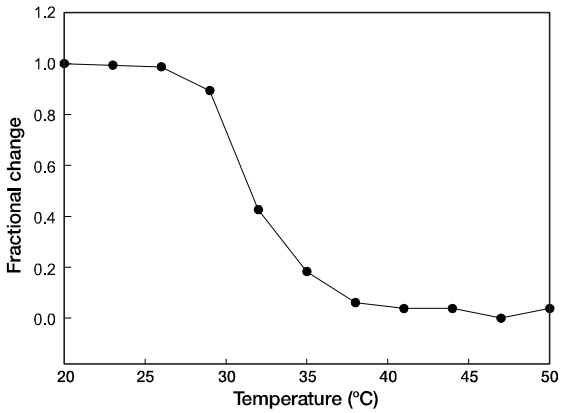

Fig. 7. Thermal denaturation curves determined by measuring the viscosity of collagen from scale of yellowfin tuna (*Thunnus albacares*) solution of 0.1 M acetic acid.

The collagen denaturation temperature (T_d) of yellowfin tuna scale collagen was 31°C, which was similar to that of calf skin collagen (32°C). Kimura et al. (1988) reported that the T_ds of collagens from carp, eel, and mackerel muscles were 32.5°C, 30.2°C, and 26.9°C, respectively, and imino acid concentrations were 43.2%, 40.2%, and 41.1%, respectively, showing that protein denaturation temperatures are higher with increasing concentrations of imino acids. Collagen denaturation temperatures of other fish were 10°C for hake, 15°C for Atlantic cod, 16.8°C for Alaska pollock, 25°C for bullhead shark, 26.5°C for Japanese seabass, and 28°C for the ocellate puffer (Nishimoto et al., 2005). The T_d for tuna collagen was higher than those of other fish collagens. One possible explanation is that tuna collagen has higher amounts of imino acids than those of other fish. Imino acids (proline and hydroxyproline), which are involved in heat stability, are important factors for industrial application.

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