Food Sci. Biotechnol. 20(1): 99-106 (2011)
DOI 10.1007/s10068-011-0014-9

RESEARCH ARTICLE

Effect of Processing Conditions on Functional Properties of Collagen Powder from Skate (*Raja kenojei*) Skins

Jinhan Shon, Ji-Hyun Eo, Su Jung Hwang, and Jong-Bang Eun

Received: 6 August 2010 / Revised: 19 October 2010 / Accepted: 3 November 2010 / Published Online: 28 February 2011

Abstract Optimum conditions for collagen extraction from skate (*Raja kenojei*) skins with various liming concentrations, extraction solution pH, extraction temperature and time, and functional properties were investigated. The optimum conditions for collagen extraction are as combination of place the skins in a lime solution of 0.15 N of NaOH, extract with 5 volumes water (pH 4.0) for 4 hr at 40°C, filter, centrifuge, and lyophilize to obtain collagen powder. The characteristics of skate skin collagen obtained under optimum extraction conditions were: solubility 82.7%, turbidity 0.28, and Hunter color *L*, *a*, and *b* values were 88.4, 0.92, and 11.2, respectively. On the other hand, the acidic pH values (3.0 and 5.0) of collagen were more resistant to precipitation upon extended heating.

Keywords: collagen, skate (*Raja kenojei*) skin, optimum condition, extraction, functional property

Introduction

Collagen is the most abundant protein of animal origin, comprising approximately 30% of total animal protein, which is generally found in skin, bone, and other connective tissues (1). Collagen has been widely used food, medicine, cosmetics, and cell cultures and the consumption has increased with development of new industrial applications (2). Collagen is a cheap and resourceful meat and fish byproduct whose main product is a gelatin that is used extensively as a food additive to increase the texture, water holding capacity and stability of several food products (3). The physicochemical and functional properties of peptides are highly influenced by their molecular structure and weight, which are greatly affected by processing conditions. The method of manufacture greatly affects the physicochemical and functional properties of the collagen. Collagen must be pretreated to convert it into a form suitable for gelatin extraction. The degree of conversion of collagen into gelatin is related to the severity of both the pretreatment and the extraction processes, which depends on pH, temperature, and extraction time (4). Fish skin is easy to hydrolyzed by acid or alkaline for its high content of soluble collagen, therefore, the pretreatment of fish skin must be mild (5). The effect of processing conditions on the properties of gelatin from skate skins with pretreatment of calcium hydroxide was reported by a previous study (6). The inconsistency in functionality can be cause by differences in gelatin or collagen sources and processing conditions. Bovine and porcine wastes are the most frequent sources of collagen. Other sources of collagen are becoming increasingly relevant, such as fish bones and skins (7). However, collagen peptide from bovine and porcine can be a food allergen. As an approach to develop new collagen and gelatin materials that have a modified allergenicity, it is considered that aquatic animal collagens could be an alternative collagen sources and is present abundantly and further processing to yield collagen can help reduce harmful environmental effects. Several studies have focused on the characterization of different fish collagens from Baltic cod (*Gadus morhus*) (8), channel

Jinhan Shon, Jong-Bang Eun (✉)
Department of Food Science and Technology, Functional Food Research Center, Chonnam National University, Gwangju 500-757, Korea
Tel: +82-62-530-2145; Fax: +82-62-530-2149
E-mail: jbeun@jnu.ac.kr

Ji-Hyun Eo
Department of Food Science and Technology, Chonnam National University, Gwangju 500-757, Korea

Su Jung Hwang
Faculty of Herbal Cuisine and Nutrition, Daegu Hanny University, Gyeongsan, Gyeonggbuk 712-715, Korea

catfish (*Ictalurus punctaus*) (1), ocellate puffer fish (*Takifugu rubripes*) (9), paper nautilus (*Argonauta argo*, Linnaeus) (10), and megrim skin (*Lepidorhombus boscil)* (4).

Skate (*Raja kenojei*) is a popular food item in South Korea. Great amounts of skate skin are disposed as waste during processing. Recently, there has been interest in investigating possible means of making more effective use of underutilized resources and industrial wastes. In this regard, skate skin has been recognized as potential food ingredients because of its excellent nutritional values and functional properties, and ability to form gels and films (11). Therefore, the objectives of this study were to investigate the optimum conditions for collagen extraction from skate skin, and to evaluate the effect of collagen on physicochemical and functional properties, including amino acid analysis, color, turbidity, gelling property (viscosity and gel strength), solubility, and heat stability.

Materials and Methods

Materials Skate skins used in the present study were obtained from a local skate processing plant (Naju, South Korea). Skate skins were immediately flash frozen in liquid nitrogen and stored at −20°C until used. All other reagents were analytical grade.

Manufacture of collagen powder The extraction procedures of collagen from skate skins were conducted according to Montero and Gomez-Guillen (4) with some modifications. The frozen skate skins were defrosted and were thoroughly washed with tap water to remove impurities. The cleaned skate skins were cut into small pieces (5×5 cm^2) to facilitate liming and soaked with 5 volumes of alkali solution (0.1 N NaOH) to remove non-collagenous proteins at a solid to solution ratio of 1:5 (v/w) for 24 hr at 4°C and washed 5 times with deionized distilled water (DDW) to remove alkali. The washed skins were extracted with 10 volumes (v/w) of 0.5 M acetic acid for 48 hr and filtered with multi layer cheesecloth. The extracts were centrifuged at 15,000 ×g for 1 hr at 4°C. The supernatants were mixed and salted out by adding NaCl to a final concentration of 5%(w/v) and centrifuged to precipitate 2 times at 15,000×g for 1 hr at 4°C. The resultant precipitate was mixed with 5 volumes (v/w) of DDW and centrifuged 2 times at 15,000×g for 1 hr at 4°C and lyophilized to obtain acid soluble collagen powder. Collagen samples were stored in sealed containers at −20°C until needed.

Proximate analysis and pH measurement The proximate analysis of collagen powder was determined in triplicate by AOAC methods (moisture, 934.01; fat, 920.39; protein, 988.05; ash, 942.05) (12). Ten g of collagen powder was homogenized with 90 mL of DDW for 30 sec using a biomixer (model 53206; Hamilton Beach, Washington, NC, USA) and pH values were measured in triplicate using a pH meter (MP120; Mettler Toledo, Schwerzenbach, Switzerland).

Yield of collagen powder The yield of collagen powder was expressed as:

% Yield
=100 (weight of dry collagen/weight of wet skate skins)

Amino acid analysis A 5 mg aliquot of collagen was dissolved in 3 mL of 6 N HCl and hydrolyzed in vacuum-sealed glass tubes at 110°C for 24 hr using a dry bath incubator (Incubator 11-718-2; Fisher Scientific Co., Fair Lawn, NJ, USA). Hydrolyzed samples were filtered through glass filters and the filtrates dissolved in citric acid buffer (pH 2.2, Sigma-Aldrich, St. Louis, MO, USA) and injected into an amino acid auto analyzer (S-433H; Sykam Co., Eresing, Germany).

Characteristics of collagen under optimal extraction condition

***Color measurement*:** Collagen powders were dispersed for 1 hr at concentrations of 6.67%(w/v) in DDW. Color values were measured using a Hunter colorimeter (CM-3500d; Minolta Co., Tokyo, Japan), which was standardized using as white blank (L=91.1, a=1.28, b= −1.54). Five readings of each collagen sample were average for color measurement. The results were expresses as Hunter L (lightness), a (redness), and b (yellowness) color values.

***Turbidity of collagen powder*:** Collagen powder was dispersed for 1 hr at concentrations of 10.0%(w/v) in DDW and the absorbance was measured at 600 nm (UV-1201; Shimadzu Co., Kyoto, Japan). The measured absorbance was expressed as the turbidity of the collagen sample.

***Viscosity of collagen powder*:** The viscosity was determined as described by Montero and Gomez-Guillen (4) with some modifications. The viscosity was determined in 10.0%(w/v) dispersions of collagen powder in DDW at different extraction conditions. Collagen dispersions were heated at 60°C and the viscosity measured with a computerized Brookfield digital viscometer (Model DV-II; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) using a No.1 spindle (Model RVT) at 60 rpm starting at 40±1°C. Viscosity of collagen powders was expressed in centipoises (cp) units.

***Gel strength*:** The gel strength was determined as described by Montero and Gomez-Guillen (4) with some modifications. The gel strength was determined in 10.0%(w/v) collagen gel in DDW at different extraction conditions. Collagen

dispersions were heated at 60°C for 16 to 18 hr and stored in refrigerator (7°C) to allow gelation. After gelling sample, the gel strength measured at 8 to 9°C on an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) with a load cell of 5 kN, cross-head speed 1 mm/sec, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger. Maximum force (g), taken when the plunger had penetrated 4 mm into the gelatin gels, are averages of 5 determinations.

***Solubility*:** The solubility of the skate skin collagen was determined according to Shon *et al*. (13) with a slight modification. A 0.5 g sample of powder was dissolved into a centrifuge tube containing 5 mL of 10 mM imidazole buffer at neutral pH 7.0. The tubes were weighed, covered with marble and allowed to stand undisturbed for 5 min after vortexing for 10 sec. The tubes were then centrifuged for 10 min at 32,000×g at 22°C. The supernatant was removed completely with a thin needle and the tubes were dried in a microwave oven (1000 W; Emerson, St. Louis, MO, USA) for 3 min and weighed. The solubility was expressed by the formula:

Insolubility (%)=100 (Insoluble sample weight/Sample weight)
Solubility (%)=100−insolubility (%)

***Heat stability*:** The heat stability of the collagen was determined using the method of Shon *et al.* (13) with some modifications. The collagen powder was vortexed (1 min) in test tubes with 4 mL of 10 mM imidazole (2%, w/v), adjusted to pH levels of 3.0, 5.0, 7.0, and 9.0 with 1 N HCl or NaOH, and sealed and heated in a thermostated water bath at 60°C±0.1 for 0, 1, 5, 10, and 20 min under mild agitation. The tubes were quickly brought to 22°C under running water, and the transmittance of visible light (600 nm) (model UV-1201; Shimadzu Co.) through the samples in cuvettes was compared with that of the unheated sample. The change in transmittance was expressed as the heat stability of the sample.

Statistical design and analysis The experiments were conducted using a one-way analysis of variance (ANOVA) with triplicate. The data were analyzed using the general linear models (GLM) procedure. Means were separated using Fisher's protected least significance test at $p<0.05$. The statistical analysis was conducted using the SAS Statistical Program, version 8.1 (SAS Institute, Cary, NC, USA) for the Windows environment (SAS) (14).

Results and Discussion

Proximate analysis and pH The proximate analysis and pH of the collagen powder extracted from skate skin under optimum conditions are shown in Table 1. Skate skin collagen had high protein content (86.4±1.57%) with a moisture content of 7.01±0.15% (Table 1). A previous report indicated that the moisture contents of collagens extracted from brownbanded bamboo shark skin was 7.77% (15). Our results showed that protein contents found in this study were somewhat lower than those of brownbanded bamboo shark skin (15). The difference in total protein was probably due to the difference in purification steps. Additionally, skate skin collagen contained a small amount of fat (0.35±0.05%) and ash content (3.38± 0.40%). Fat and ash contents were higher than those of brownbanded bamboo shark (15), but lower than those of Nile perch skin (16). Thus, the composition of collagen varies with species and might affect the extraction of collagen differently. For effective utilization of the solid byproducts for collagen, lipid and ash should be removed from the solid byproducts. Therefore, skate skin collagen appears to be a good raw material for collagen extraction because of its low ash and fat content.

Table 1. Proximate analysis and pH value of collagen powder extracted from skate skins

Moisture (%)	7.01±0.15[1)]
Total protein (%)	86.4±1.57
Fat (%)	0.35±0.05
Ash (%)	3.38±0.40
pH	7.30±0.05

[1)]Values represent mean±SD of 3 replications.

The pH of the collagen powder extracted from skate skin was 7.30±0.05 (Table 1). The pH values of the collagen from fish skins vary with fish species. The difference in pH value of the collagen may be due to the type and strength of acids employed during the extraction procedures.

Effects of NaOH concentrations For the related studies of the effects of NaOH concentrations on yields, viscosity, and gel strength of collagen, skate skins were soaked in various concentrations (0.05, 0.1, 0.15, and 0.2 N) of NaOH solution for 48 hr at 4°C. The NaOH solution was used to remove impurities (non-collagen materials and subcutaneous tissue) and to provide optimal condition for collagen extraction. The NaOH solution also used to eliminate the strong fishy odor and raise the pH. After liming, skate skins were washed with DDW (pH 7.0) for 12 hr and extracted at 50°C for 3 hr in a pH 7.0 extraction solution. The yields of collagen extracted from skate skin at different NaOH concentrations are shown in Table 2. The highest collagen extraction yield from skate skins was 10.8% at a 0.15 N, followed by 0.20, 0.10, and 0.05 N NaOH concentrations, on the basis of lyophilized dry weight. This yield of skate skin collagen was higher compared

Table 2. Effects of NaOH concentration on viscosity and gel strength of collagen extracted from skate skins

Concentration (N)	Yield (%)	Viscosity (cp)	Gel strength (g)
0.05	5.56±0.34$^{c1)}$	1,871.0±9.40^{b}	143.8±7.20^{d}
0.10	8.01±0.42^{b}	1,902.0±9.80^{b}	163.5±8.30^{c}
0.15	10.8±0.29^{a}	2,225.0±10.9^{a}	186.6±8.10^{a}
0.20	8.52±0.31^{b}	2,053.0±12.2^{ab}	171.8±9.90^{b}

[1)]Values represent mean±SD of 3 replications; [a-d]Means within the same column with different letters are significantly different (p<0.05) among NaOH concentrations.

Table 3. Effects of pH of extraction solution on viscosity and gel strength of collagen extracted from skate skins

pH	Yield (%)	Viscosity (cp)	Gel strength (g)
4.0	9.70±1.09$^{a1)}$	3,425±8.7^{a}	162.3±7.4^{c}
5.0	7.10±1.11^{b}	3,242±8.3^{ab}	168.6±6.7^{b}
6.0	7.70±0.92^{b}	2,951±7.9^{c}	185.1±7.1^{a}
7.0	7.40±0.87^{b}	2,775±10.1^{c}	182.4±8.8^{a}
8.0	8.01±0.96^{b}	3,025±9.4^{bc}	166.2±7.6^{b}
9.0	5.52±1.07^{c}	3,031±8.3^{bc}	166.2±8.1^{b}

[1)]Values represent mean±SD of 3 replications; [a-c]Means within the same column with different letters are significantly different (p<0.05) among pH values.

with results from paper nautilus outer skin (10) and cuttlefish (17), but less than bullfrog skin (18), and similar to ocellate puffer fish (9). It appears that a small amount of collagen can be obtained from skate skin compared with others and these values were about 51.4 (Japanese sea-bass), 49.8 (chub mackerel), and 50.1% (bullhead shark), respectively, on the basis of lyophilized dry weight. The difference in yield of collagen was probably due to the excessive purification steps. Previous study indicated that the yield of collagen could be increased by treating the skins first with NaOH solution at pH 11.5 for 24 hr at room temperature and then, after washing with water, with 1% H_2O_2 in 0.01 M NaOH at pH 9.3 (19). The yield of collagen can be increased by mechanical, chemical, or enzymatic pretreatments (8). One of the methods to increased the yield of collagen tissue produced by proteolytic enzymes non-specific for collagen such as pepsin, trypsin, and papain. These enzymes remove only the non-helical ends (telopeptides) of the collagen contribute remove inter-molecular cross-links, even the most stable in an acid medium (8). During enzymatic treatment of collagen tissue, not only the physicochemical properties of collagen are changed, but also non-collagen proteins are hydrolyzed. Collagen from skins of some fish species is completely soluble in acetic acid by enzymatic digestion (9,10,17).

The viscosity and gel strength of collagen extracted from skate skin at different NaOH concentrations are shown in Table 2. Viscosity is the main factor that affects physico-chemical and functional properties of a collagen and gelatin. The viscosity of collagens from skate skins with at different NaOH concentrations was small (p<0.05) (Table 2). There was little, though in some cases significant, difference in the viscosity, which varied from 1,871.0 to 2,225.0 cp for 0.05 and 0.15 N NaOH concentrations, respectively (Table 2). Viscosity of skate skin collagen was less compared with collagen from squid skins (19).

The gel strength of skate skin collagen varied with different NaOH concentrations (Table 2). The gel strength was 143.8, 163.5, 176.6, and 171.8 g in 0.05, 0.10, 0.15, and 0.20 N NaOH concentrations, respectively. The highest gel strength value observed at the 0.15 N NaOH concentrations. The gel strength of skate skin collagen was less compared with other marine fish collagens and mammalian collagens (20,21). The stability of collagen and gelatins is proportional to its total imino acid (Pro+Hyp) and glycine content (4). The gel strength is related to its imino acid and glycine content, and gelatins derived from fish collagens are weak with low melting points, compare with mammalian collagens. Mammalian collagens have a higher proportion of proline, hydroxyproline, and glycine (22). The low gel strength of skate skin collagens may be due to the lower amino acid (proline and glycine) concentrations compared with other fish and mammals (11).

Effects of pH of extraction solution Since the highest yield of skate skin collagen was obtained at limed in a 0.15 N NaOH solution, it was chosen to observe the effect of pH of extraction solution ranging from 4.0 to 9.0. After liming the sliced skate skins in 0.15 N NaOH solutions, skate skins were washed with DDW (pH 7.0) for 12 hr and collagen was extracted at 50°C for 3 hr at pH values ranging from 4.0 to 9.0. The yields of skate skin collagen varied at pH ranges of extraction solution (Table 3). The effect of pH variations on yield was highest at pH 4.0 and lowest at pH 9.0 as reflected by collagen extracted from skate skin. Our results disagreed with previous study which reported that yields increased at pH 6.0-7.0 (20).

The effects of pH variations on viscosity and gel strength in 0.5 M acetic acid are shown in Table 3. The viscosity of collagen was highest (3,425.0 cp) at pH 4.0 and decreased up to pH 7.0. It was probably due to the increase in charge repulsion at the side chain residues protein became protonated.

The gel strength of collagen was highest (185.1 g) at pH 6.0 and decreased at above pH 7.0-9.0. This might be because the molecular weight decreased slowly in the neutral region compared with alkali and acid conditions (23). Our results disagreed with previous study (24) that reported that minimum collagen viscosity observed at a pH of 6.0-8.0. A previous study indicated that apparent

Table 4. Effects of extraction temperature on viscosity and gel strength of collagen extracted from skate skins

Temperature (°C)	Yield (%)	Viscosity (cp)	Gel strength (g)
40	5.32±0.48[c1)]	3,245±8.7[a]	184.4±8.9[a]
50	7.73±0.53[bc]	3,045±9.6[b]	167.2±7.4[b]
60	9.27±0.50[b]	2,908±10.1[bc]	151.9±7.8[c]
70	11.4±0.59[a]	2,859±9.5[c]	136.5±6.7[d]

[1)]Values represent mean±SD of 3 replications; [a-d]Means within the same column with different letters are significantly different (p<0.05) among extraction temperatures.

Table 5. Effects of extraction time on viscosity and gel strength of collagen extracted from skate skins

Time (hr)	Yield (%)	Viscosity (cp)	Gel strength (g)
3	4.84±0.36[c1)]	2,134±12.1[b]	197.3±5.1[c]
4	7.61±0.48[bc]	2,368±10.9[a]	234.3±5.6[a]
5	8.47±0.42[b]	2,007±9.8[bc]	210.7±6.1[b]
6	11.5±0.51[a]	1,831±11.1[c]	183.9±5.5[d]

[1)]Values represent mean±SD of 3 replications standard deviations; [a-d]Means within the same column with different letters are significantly different (p<0.05) among extraction time.

viscosity decreased progressive pH ranging from 1.0 to 7.0 for collagen from hake skin except for pH 3.0 (25). Hydroxyproline content is important for some functional properties of collagen-derived gelatins. Rheological properties and gel strength of gelatin also increased as the amount of hydroxyproline increases (26). Previous report also suggested that the gel strength may be dependent on the isoelectric point (pI) and may also be controlled, to a certain extent, by adjusting the pH (21). Gelation occurs when native globular collagen proteins are denatured (unfolded) in the presence of heat. Generally, gels formed with heating at low pH (6.0) are more coagulated and less elastic than gels formed at pH 7.0 to 9.0. For many collagens, minimum viscosity is at pH value of 6.0-8.0 (24). Collagens have a minimum viscosity at the pI range. Thus, the viscosity improved when acid soluble collagen was adjusted to pH values of 2.0-3.0. The pI region of collagen is pH values of 6.0-9.0 (27).

Effects of extraction temperature Since the highest yield of collagen was obtained at limed in a 0.15 N NaOH solution and at pH 4.0, it was chosen to observe the effect of temperatures ranging from 40 to 70°C. After liming the sliced skate skins in 0.15 N NaOH solutions, skate skins were washed with DDW (pH 7.0) for 12 hr and collagen was extracted at temperatures ranging from 40 to 70°C for 3 hr at pH 4.0. The extraction yields of skate skin collagen at various temperatures are shown in Table 4. The highest extraction yield (11.4%) of collagen from skate skin observed at extraction temperature of 70°C, followed by 60, 50, and 40°C, on the basis of lyophilized dry weight. Results indicated that the yields of skate collagen increased as extraction temperature increased.

Collagen viscosity was measured at varied extraction temperatures (40-70°C) (Table 4). Viscosity of collagen skin at 40°C was highest values of 3,245.0 cp compared with other temperatures (Table 4). Heating the collagen caused to decrease viscosity. Our results agreed with previous study which reported that the gel forming ability and the physical properties decreased at temperature higher than 50°C due to breakage of hydrogen bonds and free amino acid hydroxyl groups (16). Viscosity of skate skin collagen decreased continuously on extraction temperature up to 70°C. Previous reports indicated that megrim skin collagen showed higher viscosity at 25°C compared to 40 and 60°C (4) and viscosity of bigeye snapper (28) and yellowfin tuna skin collagen (2) decreased continuously on heating up to 30°C and decreased slowly in the range of 30-50°C. Our result agreed with reported by previous studies (1,4,28). From the result, similar changes in viscosity of skate skin collagen caused by heat-treatment were observed. Denaturation of collagen structure caused by heat-treatment is associated with the changes in viscosity (9). Denaturation temperatures of collagens from the skins of Alaska pollack (16.8°C) and Japanese sea bass, chub mackerel, bullhead shark, and ocellate puffer fish ranged from 25.0 to 28.0°C and carp, yellow sea bream, and Japanese sea bass fin ranged from 29.5 to 31.7°C (9).

The gel strength of skate skin collagen at 40°C was highest values of 184.4 g compared with other temperatures (Table 4). Results showed that the gel strength decreased as temperatures increased in the ranged from 50 to 70°C. Physicochemical properties decreased at extraction temperatures higher than 40°C due to hydrolysis of cross-linkages in collagen and other proteins (20).

Effects of extraction time Since the highest yield of collagen was obtained at limed in a 0.15 N NaOH solution and at pH 4.0, it was chosen to observe the effect of extraction times ranging from 3 to 6 hr. After liming the sliced skate skins in 0.15 N NaOH solutions, skate skins were washed in DDW (pH 7.0) for 12 hr and collagen was extracted at 50°C for 3 hr at pH 4.0. The yields of collagen from skate skin increased with increasing extraction time up to 6 hr (Table 5). The highest yield of collagen observed at extraction time of 6 hr (11.5%). Data indicated that the yield of collagen increased as extraction time increased in the ranged from 4 to 6 hr. A previous study found that yields of collagen slightly increased with increasing extraction times (20). The yield of collagen extraction from the skin of Baltic cod in 0.5 M acetic acid with same concentration of pepsin increased the yield of collagen extracted after 72 hr increased by about 45% in comparison

Table 6. Amino acid composition of collagen extracted from skate skins (residues/1,000 residues)

Amino acid	
Hydroxyproline	76.0±7.1[1)]
Aspartic acid	39.0±2.3
Threonine	34.0±1.6
Serine	48.0±2.4
Glutamic acid	78.0±2.3
Proline	86.0±2.9
Glycine	334.0±9.7
Alanine	109.0±7.4
Cystine	1.00±0.05
Valine	25.0±1.7
Methionine	15.0±1.4
Isoleucine	17.0±1.3
Leucine	25.0±1.8
Tyrosine	4.00±0.9
Phenylalanine	13.0±1.6
Hydroxylysine	8.00±1.1
Histidine	10.0±1.2
Lysine	26.0±2.0
Arginine	52.0±2.3
Total	1,000

[1)]Values represent mean±SD of 3 replications.

with the 24 hr digestion (8). A previous study indicated that the yield of collagen increased in presence of enzyme (pepsin) and a longer reaction time rendered a higher yield (7). However, the extracted collagen in the absence of pepsin was mainly acid solubilized collagen, which contributed more solubilized with increasing extraction times (4).

The physicochemical properties of collagen extracted from skate skins at various extraction times are shown in Table 5. The viscosity was the highest values (2,368.0 cp) at 4 hr of extraction time compare with other extraction times (Table 5). One physicochemical characteristics of collagen is high viscosity. The high viscosity can be accounted for by the high proportion of β- and γ-chains, resulting in a higher average molecular weight (29). Determination of the apparent viscosity seems to be a suitable method for determining collagenous material functionality.

The highest gel strength (234.3 g) was observed at 4 hr of extraction time (Table 5). Both viscosity and gel strength increased up to 4 hr extraction time and decreased to 6 hr extraction time. Gelation property is one of the numerous desirable functional attributes of food proteins.

Amino acid composition The amino acid composition of acid soluble collagen extracted from skate skin, expressed as residues/1,000 total residues, is presented in Table 6. Glycine was the most abundant amino acid in skate skin collagen and there were relatively high contents of alanine, proline, glutamic acid, and hydroxyproline, decreasing in that order. Glycine accounted for more than 30.0% of all amino acids in skate skin collagen. Its value was approximately 334.0 residues, significantly higher than that of channel catfish (1). On the contrary, cysteine, methionine, and tyrosine are low just like many fish species collagen. The total imino acid (hydroxyproline+proline) content of skate skin collagen was about 16%, which was more than some reports for collagen from cold-water fish species, but lower than that of cuttlefish skin (17), and similar to that of bullfrog skin (18), whereas that of yellowfin tuna dorsal skin (2) and bigeye snapper skin (3) was about 21.0 and 21.0%, respectively. The difference in imino acid content among animals was associated with the difference in the living environments of their sources, particularly habitat temperature. Additionally, the imino acid content was reported to have a major influence on thermal stability of collagen (16). The stability of collagen was proportional to the total content of pyrrolidine imino acids. The Pro+Hyp rich zones of the molecules are most likely involved in the formation of junction zones stabilized by hydrogen bonding (22). Hydroxyproline is important in maintaining the stabilization of the trimers in collagen (1). Rheological properties and gel strength of gelatin increased as the amount of hydroxyproline increased (26).

Table 7. Characteristics of collagen extracted from skate skins under optimal extraction condition

Solubility (%)	Turbidity	Hunter color value		
		L	a	b
82.7±1.87[1)]	0.28±0.04	88.4±0.09	0.92±0.04	11.2±0.06

[1)]Values represent mean±SD of 5 replications.

Characteristics of collagen under optimal extraction condition

***Color measurements*:** The Hunter color values of collagen powder extracted from skate skin varied with pretreatment of collagen (Table 7). The Hunter color *L* value, which reflects lightness, was affected by the extraction solution, was 88.4 for collagen sample, indicating good visual whiteness. The skate skin collagen had a white or slightly yellow color, which is a positive attribute. In respect to color the collagen is equal to commercial products. Therefore, collagen powder from skate skin, if added in food products, is not likely to negatively impact the color of final products. The other relevant part of the Hunter color *a* value, which represents redness, was also affected by the extraction solution, was 0.92 for collagen samples. The Hunter color *b* value (yellowness) was 11.2 for collagen samples. The Hunter color *b* value of skate skin collagen increased as extraction time increased. A difference in the color values at different extraction

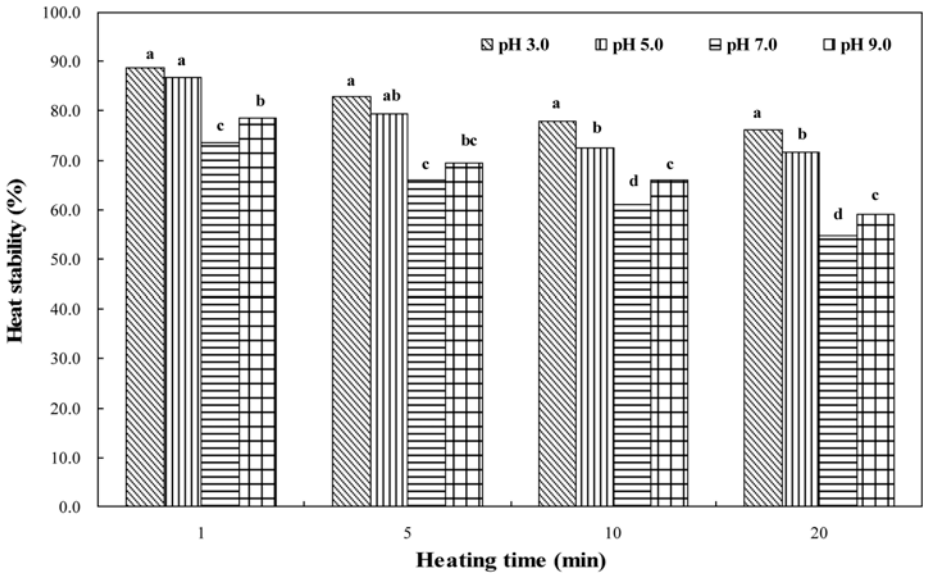

Fig. 1. Heat stability of native collagen powder (2%, w/v) at different pH levels in imidazole-HCl buffer at 60ºC. Different letters above bars indicate significant difference ($p<0.05$) within the pH group.

solution is due to the protein denaturation and structural change by hydrolysis. One of the most important features of collagen, with regard to its use, is color (8). Collagen entirely devoid of color is difficult to obtain because of the presence of pigment in fish skins. The most effective way to separate the pigments from collagen is to extract collagen directly from skate skins with organic acids. Leaching of skate skins with 0.5 M acetic acid at 4ºC for 24 hr, followed by homogenization and centrifuging, leads to a colorless collagen solution. The color of the gelatin does not influence other functional properties (22).

***Turbidity of collagen powder*:** Turbidity of skate skin collagen dispersion at concentrations of 10.0%(w/v) in distilled water is determined (Table 7). Turbidity of collagen extracted from skate skin was considerably low value of 0.28±0.04 (Table 7). Turbidity value reflects concentration of residual lipid and other colloidal material that were present in collagen. The total fat content of collagen was 0.35±0.05, as determined immediately after preparation of the lyophilized powder from freshly separated collagen. Turbidity increased with concentration for collagen dispersions increased (data not shown). Turbidity values are largely dependent on efficiency of the clarification (filtration) process. The turbidity was also due to protein-protein aggregation leading to the formation of particles larger than the wavelength of light. Lower turbidity of collagens indicates greater dispersibility. Heating the collagen dispersion at 60ºC for 30 min might be increased turbidity due to protein aggregation (4). Residual lipid in collagen has been recognized as being detrimentally impacted emulsifying and foaming properties and flavor qualities. Therefore, removal of residual lipids from collagen resulted in improves functionalities such as emulsifying and foaming properties. In other case, previous reports indicated that turbidity of gelatin from skate skins was lower compared with Alaska pollack skin, but was much greater compared with commercial bovine skin (20). The higher turbidity in skate collagen reflects its poorer quality compared with commercial collagens.

***Solubility*:** The effect of neutral pH 7.0 on solubility of collagen from skate skin is shown in Table 7. The solubility of skate skin collagen was 82.7±1.87% at neutral pH. Generally, solubility of collagens was more soluble in acidic pH ranges of 2.0-5.0 with relative solubility greater than 80.0% and decrease sharply in solubility at the neutral pH. However, different collagens had varying solubility at pH ranging from 6.0 to 10.0. The variation in solubility of proteins with pH might be due to differences in pI. The pI region of collagen is pH values of 6.0-9.0 (27). At acidic pH of 1.0, collagen might undergo denaturation to some extent, leading to decrease solubility. As pHs are being in the pI range of collagen, the molecular charges became diminished and decreased in solubility occurred (30). Thus, the lowest solubility of collagens was observed at pH around 7.0. The differences in pH maxima for solubility between collagens from skin and bone might be due to different molecular properties and conformations between the collagen. Collagen from skin might possess a lower degree of molecular cross-linking and weaker bonds compared with collagen from bone (28).

***Heat stability*:** The heat stability is a desirable attribute for food proteins, especially for high protein beverages that need heat treatments (13). The heat stability of the skate skin collagen was studied at different pH levels and heating

times (Fig. 1). At the pH values of 3.0, 5.0, 7.0, and 9.0, after 1 min of heating, skin collagen showed the highest heat stability values at 88.7, 86.8, 73.6, and 78.7%, respectively (Fig. 1). However, these heat stability values decreased drastically after further heating for 20 min. The heat stability decreased by 16.0 and 20.2% (in contrast to 1 min) at the acidic pH levels of 3.0 and 5.0, respectively, as compared to 34.0 and 32.1% for pH levels of 7.0 and 9.0, respectively (Fig. 1). This indicates that the acidic pH values of collagen were more resistant to precipitation upon extended heating. This heat resistance property would be useful in foods such as health and sports drinks, which are acidic in nature and must be pasteurized, and in other foods that have to be retort stable. The higher heat stability of the acidic pH values of collagen than that of the neutral or high pH values of collagen may be due to differences in their compositional or physicochemical properties; in particular, pH and ash content. Collagen also contains a high level of calcium, resulting from the solubilization of colloidal calcium phosphate at low pH values, which may influence the heat stability of collagen (13).

In conclusion, skate skin collagen had similar physicochemical and functional properties and higher yields compared with collagens produced from other marine fish skins. Future research is needed to improve functional properties of collagens produced from skate skins. Skate skin, which is presently considered to be a waste product, could be effectively used as natural gelatin and collagen sources if thermal stability of the collagen is improved.

Acknowledgments This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF 351-2009-1-C00084).

References

1. Liu HY, Ding L, Guo SD. Studies on collagen from the skin of channel catfish (*Ictalurus punctaus*). Food Chem. 101: 621-625 (2007)
2. Woo J-W, Yu S-J, Cho S-M, Lee Y-B, Kim S-B. Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin. Food Hydrocolloid 22: 879-887 (2008)
3. Jongjareonrak A, Benjakul S, Visessanguan W, Tanaka M. Isolation and characterization of collagen from bigeye snapper (*Priacanthus macracanthus*) skin. J. Sci. Food Agr. 85: 1203-1210 (2005)
4. Montero P, Gomez-Guillen MC. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. J. Food Sci. 65: 434-438 (2000)
5. Liu HY, Ding L, Guo SD. Extraction and properties of gelatin from channel catfish (*Ictalurus punctaus*) skin. Lebensm. -Wiss. Technol. 41: 414-419 (2008)
6. Cho S-H, Jahncke ML, Chin K-B, Eun J-B. The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins. Food Hydrocolloid 20: 810-816 (2006)
7. Nalinanon S, Bebjakul S, Visessanguan W, Kishimura H. Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). Food Chem. 104: 593-601 (2007)
8. Skierka E, Sadowska M. The influence of different acids and pepsin on the extractability of collagen from the skin of Baltic cod (*Gadus morhua*). Food Chem. 105: 1302-1306 (2007)
9. Nagai T, Araki Y, Suzuki N. Collagen of the skin of ocellate puffer fish (*Takifugu rubripes*). Food Chem. 78: 173-177 (2002)
10. Nagai T, Suzuki N. Preparation and partial characterization of collagen from paper nautilus (*Argonauta argo*, Linnaeus) outer skin. Food Chem. 76: 149-153 (2002)
11. Cho S-H, Jahncke ML, Eun J-B. Nutritional composition and microflora of the fresh and fermented skate (*Raja kenojei*) skins. Int. J. Food Sci. Nutr. 55: 45-51 (2004)
12. AOAC. Official Methods of the AOAC. 15th ed. Association of Official Analytical Chemists, Washington, DC, USA (1995)
13. Shon J, Lee S-H, Lee F-Z, Lee B-D, Eun J-B. The effect of heating on the physicochemical and functional properties of acid whey compared to sweet whey. Food Sci. Biotechnol. 16: 836-842 (2007)
14. SAS Institute, Inc. SAS User's Guide. SAS Companion for the Microsoft Windows Environment (version 8.1). Statistical Analysis Systems Institute, Cary, NC, USA (2001)
15. Kittiphattanabawon P, Benjakul S, Visessanguan W, Kishimura H, Shahidi F. Isolation and characterisation of collagen from the skin of brownbanded bamboo shark (*Chiloscyllium punctatum*). Food Chem. 119: 1519-1526 (2010)
16. Muyonga JH, Cole CGB, Duodub KG. Extraction and physicochemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloid 18: 581-592 (2004)
17. Nagai T, Yamashita E, Taniguchi K, Kanamori N, Suzuki N. Isolation and characterisation of collagen from the outer skin waste material of cuttlefish (*Sepia lycidas*). Food Chem. 72: 425-429 (2001)
18. Li H, Liu BL, Gao HL, Chen HL. Studies on bullfrog skin collagen. Food Chem. 84: 65-69 (2004)
19. Kolodziejska I, Sikorski ZE, Niecikowska C. Parameters affecting the isolation of collagen from squid (*Illex argentinus*) skins. Food Chem. 66: 153-157 (1999)
20. Kim S-K, Byun H-G, Lee E-H. Optimum extraction conditions of gelatin from fish skins and its physical properties. J. Korean Ind. Eng. Chem. 5: 547-559 (1994)
21. Gudmunsson M, Hafsteinsson H. Gelatin from cod skin as affected by chemical treatments. J. Food Sci. 62: 37-39 (1997)
22. Johnston-Banks FA. Gelatin. pp. 258-259. In: Food Gels. Harris P (ed). Elsevier Applied Science Publishing Co., New York, NY, USA (1990)
23. Grossman S, Bergman M. Process for the production of gelatin from fish skin. U.S. Patent 5, 093. 474 (1992)
24. Stainsby G. Viscosity of diluted gelatin solutions. Nature 169: 662-665 (1952)
25. Montero P, Gomez-Guillen MC, Borderias AJ. Functional characterization of muscle and skin collagenous material from hake (*Merluccius merluccius* L.). Food Chem. 65: 55-59 (1999)
26. Gilsenan PM, Ross-Murphy SB. Rheological characterization of gelatins from mammalian and marine sources. Food Hydrocolloid 14: 191-195 (2000)
27. Foegeding E, Lanier TC, Hultin HO. Characteristics of edible muscle tissue. pp. 879-942. In: Food Chemistry. Fennema OR (ed). Marcel Dekker, New York, NY, USA (1996)
28. Kittiphattanabawon P, Benjakul S, Visessanguan W, Nagai T, Tanaka M. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chem. 89: 363-372 (2005)
29. Ogawa M, Portier RJ, Moody MW, Bell J, Schexnayder MA, Losso JN. Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). Food Chem. 88: 495-501 (2004)
30. Montero P, Jimennez-Colmenero F, Borderias J. Effect of pH and the presence of NaCl on some hydration properties of collagenous material from trout (*Salmo irideus* Gibb) muscle and skin. J. Sci. Food Agr. 54: 137-146 (1991)