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Characterization of Acid-soluble Collagen from Alaska Pollock Surimi Processing By-products (Refiner Discharge)

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Abstract The study was carried out to examine on the refiner discharge from Alaska pollock as a collagen resource by characterizing biochemical and functional properties of collagen. The refiner discharge from Alaska pollock surimi manufacturing was a good resource for collagen extraction according to the results of total protein, heavy metal, volatile basic nitrogen, collagen content, amino acid composition, and thermal denaturation temperature (TDT). TDT of acid soluble collagen from refiner discharge showed 20.7°C, which was similar to that of collagen from Alaska pollock muscle and was higher than that of collagen from Alaska pollock skin. TDT of acid-soluble collagen from refiner discharge was, however, lower than those of skin collagens from warm fish and land animal. Acid-soluble collagen from refiner discharge of Alaska pollock could be used as a functional ingredient for food and industrial applications according to the results of water and oil absorption capacities, and emulsion properties. In addition, if the thermal stability of the acid-soluble collagens is improved, collagen from refiner discharge from Alaska pollock could be more effectively used.

Keywords: surimi by-product, refiner discharge, collagen, Alaska pollock, surimi

Introduction

Refiner discharge, which is a solid by-product of surimi manufacturing, has received increased attention as potential gelatin and collagen resources. Refiner discharge is about 4-8% of whole fish and contains a significant amount of collagen (1) (Fig. 1). Based on the USA annual surimi production (220,000 to 240,000 MT), 10,000 to 20,000 MT of crude collagen can be generated from a refiner discharge (2). Conventionally, most refiner discharge is, however, used to produce fish meal and fertilizer or is directly discharged into estuaries, resulting in environmental pollution and offensive odors. From the viewpoint of environmental protection, as well as utilization of limited marine bioresources, it is important to development a preparation method of fish collagen from refiner discharge for making more effective use of this under-utilized resource or waste material.

Collagen is used in various food applications (clarification agent, emulsifier, or whipping agent) (1, 2). Its usage extends even further to other industrial (shampoo and lipstick) and pharmaceutical applications (film-forming agent, microencapsulation, or tablet coating) (3). However, it is commonly obtained from land animal by-products. Today's health-conscious consumers have a negative attitude toward collagen extracted from land animals because of the recent outbreaks of bovine spongeform encephalopathy (BSE), foot and mouth disease, and chicken flu (4). Refiner discharge is mainly composed of tendon separated while concentrating myofibrillar proteins from thoroughly washed mince (2). For the reason, consumers recognize that refiner discharge is separated from edible muscle tissue, not from inedible waste like fish skins or frame which is mixed with guts and blood. Therefore, it may be used for the production of consumer-friendly collagen. It is, however, generally known that cold-water fish gelatins are characterized by having considerably lower denaturation temperature (denaturation temperature, 15-33°C) than gelatins from warm blooded animals (denaturation temperature, 40°C), moreover, the gels are also softer or can not be formed, which is directly related to the fact that imino acid (proline and hydroxyproline) content is higher in the latter (5-7). The result suggests that fish collagen or gelatin cannot be used in products demanded heat-reversible properties of gelatin because gelatin gel melts at around room temperature. To use collagen from fishery products in various foods or other industrial or pharmaceutical applications, their functional properties (water, oil absorption, and emulsion ability and stability) as well as physicochemical properties must be examined.

Some efforts have been made toward the preparation of collagens from fishery products, such as fish skin (8, 9), fish bone (3), and refiner discharge from Pacific whiting (1). However, these researchers only used fillet by-products as a resource for extracting collagen except for research of Kim and Park (1) used refiner discharge from Pacific whiting. So far, little information regarding the extraction and the characteristics of collagen from Alaska pollock refiner discharge has been reported.

The objective of this study was to examine refiner discharge, a solid by-product, from Alaska pollock surimi processing as a collagen resource by characterizing biochemical and functional properties of collagen. For comparison, the collagens from skin and muscle of Alaska pollock were also examined on biochemical and functional properties.

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Materials and Methods

Materials Alaska pollock (*Theragra chalcogramma*), 61 to 71 cm in body length, was caught off the coast of Alaska by trawl. Alaska pollock and its surimi by-products were obtained from a commercial surimi processing plant (Traident Ltd., Kodiak, AK, USA) in March 2005.

Skin from Alaska pollock was generated from the deboning and mincing steps, whereas refiner discharge was seperated from the refining steps immediately before the screw-press dewatering. The samples (fresh whole fish and solid by-products of Alaska pollock surimi processing) were immediately frozen, transferred in keeping frozen to the Gyeongsang National University, Seafood By-products Lab., and kept frozen at -30°C until used for collagen extraction.

Proximate composition, volatile basic nitrogen, and heavy metal According to AOAC methods (10), moisture content was quantified by oven drying at 105°C, total protein by the Kjeldahl procedure, and crude ash by incineration in a muffle furnace at 550°C. In addition, total lipid was extracted into a methanol-chloroform mixture and quantified according to the method of Bligh and Dyer (11). The concentration of volatile basic nitrogen (VBN) was determined by using the method of Conway (12). The mercury content was determined by the combustion gold amalgamation method (13) using a mercury analyzer (SP-3A; Nippon Instrument Co., Tokyo, Japan). Other heavy metals, such as Pb, Cd, and Cr, were determined by the wet ash method (14), using an inductively coupled plasma spectrophotometer (ICP, Atomscan 25; Thermo Electron Co., Waltham, MA, USA).

Preparation of collagen fractions All analyses were performed in a cold room (5°C). Native collagen was prepared as described by Sato *et al.* (15) and Nagai and Suzuki (3). Alaska pollock whole muscle and its surimi solid by-products were first cut into small pieces before homogenizing with 5 volumes (v/w) of cold distilled water. The homogenate was then centrifuged at 10,000×g for 20 min. To the residues, 20 volumes (v/w) of 0.1 N NaOH was added to remove noncollagenous protein. The homogenate was stirred overnight before centrifuging at 10,000×g for 20 min. Alkali-extractions were repeated 3 additional times. Final precipitate was washed thoroughly with cold distilled water. To all the residues, 10 volumes (v/w) of 0.5 M acetic acid was added. Suspensions were stirred for 3 days before centrifuging at 10,000×g for 20 min. This acid extraction process was repeated once more. The precipitates were then washed with cold distilled water at a 1:2 (w/v) ratio. The supernatant from acid extraction and the filtrate from rinsing were combined and subjected to salting out by adding NaCl to 2.0 M before centrifuging at 20,000×g for 20 min. The precipitate was redissolved in 0.5 M acetic acid and then dialyzed (molecular weight cut-off 10,000) against cold distilled water to remove salt. Salting out and dialysis were repeated twice more for further purification of collagen. The final dialyzed solution was used as acid-soluble collagen fraction.

Separately from preparation of acid-soluble collagen, centrifuge residues obtained after acid extraction were heated with 5 volumes (v/w) of distilled water in an autoclave at 120°C for 1 hr and then centrifuged at 10,000 ×g for 20 min. The precipitates were rinsed using hot distilled water at a 1:2 (w/v) ratio. The supernatant from centrifugation and the filtrate from rinsing were combined and used as acid-insoluble collagen fraction. The acid-insoluble fraction was used for calculation of total collagen N concentration.

Acid-soluble fraction was lyophilized and used for further analyses of collagen characteristics. Concentration and solubility of collagen were calculated according to the following equations:

$$\text{Collagen concentration (\%)} = (\text{TCN}/\text{TN}) \times 100$$
$$\text{Collagen solubility (\%)} = (\text{ASCN}/\text{TCN}) \times 100$$

TCN (%) = total collagen-N concentration
TN (%) = total-N concentration
ASCN (%) = acid-soluble collagen-N concentration

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) SDA-PAGE was performed by the method of Laemmli (16) using 7.5% running gel containing 0.1% SDS at pH 8.8, and 5% stacking gel. SDS-PAGE was performed using a slab gel electrophoresis system (FB-VE 16-1; 16×14 cm, Fisher Scientific, Pittsburgh, PA, USA). Protein samples and protein marker (M 4038; Sigma Chemical Co., St. Louis, MO, USA) were heated at 100°C for 3 min in 10 mM tris-HCl buffer (pH 6.8) containing 2% SDS, 1% 2-mercaptoethanol, 25% glycerol, and 0.1% bromophenol blue. The gels were stained for protein with 0.1% Coomassie brillant blue R-250 and destained in 10% methanol and 10% acetic acid.

Amino acid composition Amino acid composition was determined using an amino acid analyzer (Biochrom 20; Parmacia Biotech., Little Chalfont, England) according to the method of Kimura *et al.* (17). Samples were hydrolyzed in 6 N HCl in evacuated/sealed tubes at 110°C for 16 hr. The hydrolysates was evaporated to dryness in a vacuum evaporator at 40°C and then diluted with Li^+-citrate buffer for analyses of amino acids containing hydroxyproline and hydroxylysine.

Hydroxylation (%) of proline (Pro) and lysine (Lys) was calculated based on the amino acid composition according to the following equations:

$$\text{Hydroxylation of Pro (\%)} = (\text{Hyp} \times 100)/(\text{Pro} + \text{Hyp})$$
$$\text{Hydroxylation of Lys (\%)} = (\text{Hyl} \times 100)/(\text{Lys} + \text{Hyl})$$

Hyp = hydroxyproline, Hyl = hydroxylysine.

Thermal denaturation temperature Thermal denaturation temperature (TDT) was performed using the method of Zhu and Kimura (18). The Ostwald viscometer was filled with 5 mL of collagen solution (30 mg collagen dissolved in 100 mL acetic acid solution, 0.1 M) or 0.1 M acetic acid as a control sample. After immersing viscometer in the water bath at 8°C, it was kept for 30 min to allow the collagen solution to equilibrate to the water bath temperature. The temperature was increased stepwise up

to 45°C and maintained at each temperature (15 to 31°C at every 2°C and at 45°C) for 30 min. The efflux time of the solution incubated in the water bath was checked in the tube of an Ostwald viscometer and then specific viscosity (η_{sp}) was calculated according to the following equation:

$$\text{specific viscosity } (\eta_{sp}) = (t\text{-}to)/to$$

t = efflux time of the collagen solution
to = efflux time of the 0.1 M acetic acid

We assumed that collagen helical conformation was undenaturated at 8°C, whereas breakdown was completed at 45°C (18).

TDT of collagen solution was defined as the temperature at which the change in viscosity reached by 50%. Fraction change was calculated as follows:

$$\text{Fraction change} = [(E_2/C) - (E_3/C)]/[(E_1/C) - (E_3/C)]$$

C=collagen concentration (mg/mL); E_1=specific viscosity at 8°C; E_2=specific viscosity at measured temperature (°C); E_3=specific viscosity at 45°C.

Functional properties Water and oil absorption capacities were determined by the method of Beuchat (19). Freeze-dried collagen (0.3 g) was mixed with 10 mL distilled water for water absorption measurement and with 10 mL soybean oil for oil absorption measurement. Mixing was done at fast speed using a vortex mixer for 30 sec. Samples were then allowed to stand at room temperature (22°C) for 30 min before centrifuging at 5,000 ×g for 30 min. The volume of supernatant was measured in a graduated cylinder (10 mL), and each capacity was expressed as (10 mL − measured volume)/collagen weight (0.3 g).

Emulsifying activity and cooking stability were determined by the method of Wang and Kinsella (20). Freeze-dried collagen (0.2 g) and Tween-80 (0.2 g, Fisher Scientific) were added to 20 mL of 0.1 N acetic acid, respectively, and the mixture was set at room temperature for 2 min using a PT 10/35 polytron homogenizer at setting 3 (Kinematica, Luzern, Switzerland) for generating medium speed. Twenty mL of vegetable oil (soybean oil) were added before mixing for 3 min using a PT 10/35 polytron homogenizer at high speed. The resulting emulsion was centrifuged at 1,300×g for 15 min. Emulsifying activity was expressed as the following equation:

(Height of emulsified layer/height of total contents in the tube) × 100

Cooking stability was determined similarly to the emulsifying activity except that the emulsion in the centrifuge tube (height × i.d. 11.5×3.0 cm) was initially heated in a water bath (80°C) for 30 min and subsequently cooled to 15°C before centrifuging at 1,300×g for 15 min. Cooking stability was measured using the following equation:

(height of emulsified layer after centrifugation/height of total contents in the tube) × 100%

Statistical analysis Statistical analysis was done using the ANOVA (analysis of variance) test. Significant differences of means were performed using Systat Version 7.5K (SPSS, Inc., Richmond, VA, USA) at $p<0.05$ (21).

Results and Discussion

Proximate composition Proximate composition of solid by-products and whole muscle of Alaska pollock are shown in Table 1. Moisture content was in the descending order of refiner discharge (84.7%), muscle (80.9%), and skin (71.3%) of Alaska pollock. The highest moisture in refiner discharge was because the refiner discharge is separated from myofibrillar protein in washing process. Regardless of the kinds of samples, such as solid by-products and whole muscle of Alaska pollock, crude lipid, and ash of all samples, which are foreign components for the effective utilization of collagen, showed a significantly low concentration (less than 1%). The highest crude protein was obtained from skin sample, followed by whole muscle, and refiner discharge. There was, however, no difference, in the crude protein composition (88.2-90.9%) on the dried weight basis among samples. Kim and Park (1) also reported that the major component of skin, refiner discharge, and whole muscle of Pacific whiting was crude protein, ranging from 82.8 to 88.5%.

Volatile basic nitrogen and heavy metal Volatile basic nitrogen (VBN) and heavy metal contents of solid by-products, such as skin and refiner discharge, and whole muscle of Alaska pollock are shown in Table 2. VBN contents in solid by-products were 19.6 mg/100 g for the skin and 7.2 mg/100 g for the refiner discharge. The

Table 1. Proximate composition of various solid by-products and muscle of Alaska pollock[1)] (g/100 g)

	Muscle	Solid by-products	
		Skin	Refiner discharge
Moisture	80.9±0.2	71.3±0.8	84.7±0.1
Crude lipid	0.4±0.2 (2.1)[2)]	0.9±0.1 (3.1)	0.2±0.1 (1.3)
Crude protein	17.1±0.1 (89.5)	26.1±0.1 (90.9)	13.5±0.2 (88.2)
Crude ash	0.8±0.2 (4.2)	1.0±0.1 (3.5)	1.0±0.3 (6.5)

[1)]Values are the means±SD of 3 determinations.
[2)]The value in the parenthesis means g/100 g of dry material.

Table 2. Volatile basic nitrogen (VBN) and heavy metal contents of various solid by-products and muscle of Alaska pollock[1)]

Components		Muscle	Solid by-products	
			Skin	Refiner discharge
VBN (mg/100 g)		17.5±0.9	19.6±1.0	7.2±0.9
Heavy metal (mg/kg)	Chromium	ND[2)]	0.06±0.02	0.08±0.03
	Lead	ND	ND	0.03±0.01
	Cadmium	ND	ND	ND
	Mercury	ND	ND	ND

[1)]Values are the means±SD of 3 determinations.
[2)]Not detected.

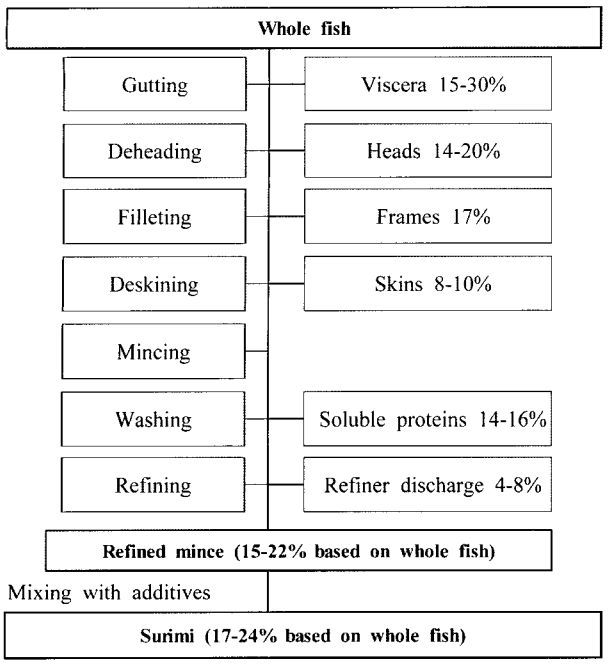

Fig. 1. Surimi processing flow chart with mass balance.

concentrations of VBN in skin and refiner discharge were lower than 20 mg/100 g, which are believed to be an acceptable limit for marine products (22). Cadmium and mercury were not detected in all samples. Lead was also not detected in whole muscle and skin except for refiner discharge (0.03 mg/kg). Chromium was found to be 0.06 mg/kg for skin and 0.08 mg/kg for refiner discharge. According to Codex Code (23), the heavy metal safety values were 0.2 to 1.0 mg/kg for cadmium, 0.2 to 0.4 mg/kg for lead, and zero for mercury and cadmium. Because the concentration of VBN and heavy metals in solid products were below these reported safety value limits, skin and refiner discharge appeared safe as a raw material for collagen.

Collagen content Collagen contents of solid by-products and whole muscle of Alaska pollock are shown in Fig. 2. Collagen content of refiner discharge was 28.8%, which was much lower than that (57.1%) of skin. Kim and Park (1) reported that collagen content of refiner discharge of Pacific whiting surimi by-products (55.3%) was slightly lower than that of skin (65.6%). The difference in collagen content between refiner discharges of Pacific whiting and Alaska pollock was considered to be due to the difference in the number of washing for separating connective tissue from myofibrillar protein as well as difference of fish species. The collagen concentration of whole muscle showed a significant lower (1.2%) than those of solid by-products of Alaska pollock surimi. A significant difference in collagen content between whole muscle and refiner discharge was probably because refiner discharge is mainly composed of tendon. Park *et al.* (24) reported that collagen contents of fish muscles ranged from 0.34 to 2.19% (sardine, 0.34%; rainbow trout, 0.47%; mackerel, 0.50%; jack mackerel, 0.51%; carp, 0.60%; filefish, 0.68 %; snapper, 0.73%; sea bass, 0.88%; common mullet, 1.16 %; flatfish, 1.42%; common eel, 1.99%; sharp toothed eel, 1.54%; conger eel, 2.19%).

Collagen solubility The solubilities of collagens from solid by-products and whole muscle of Alaska pollock are shown in Fig. 3. The solubility of collagen was 62.9% for muscle, 71.5% for skin, and 60.3% for refiner discharge. There was, however, no difference ($p>0.05$) in solubility between collagens from refiner discharge and whole muscle. The result might be due to refiner discharge is separated while concentrating myofibrillar proteins from thoroughly washed mince. Montero *et al.* (8) and Yamaguchi *et al.* (25) also reported that collagen solubility of hake (*Merluccius merluccius* L.) was higher in skin collagen (93.1 and 65.2%, respectively) than in muscle collagen (74.4 and 60.0%, respectively). A difference in solubility between our results and values of the literatures was believed to be due to a difference in caught area, age

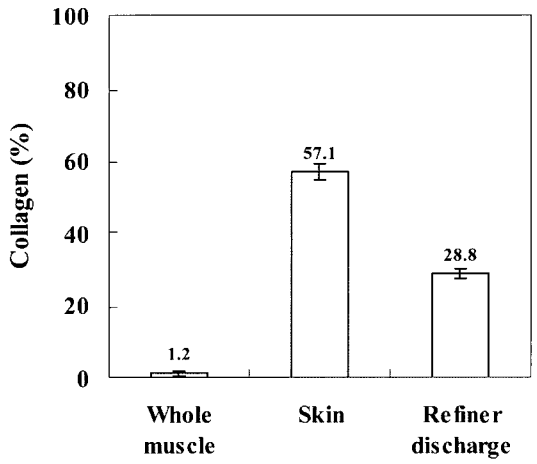

Fig. 2. Collagen contents of various solid by-products and muscle of Alaska pollock.

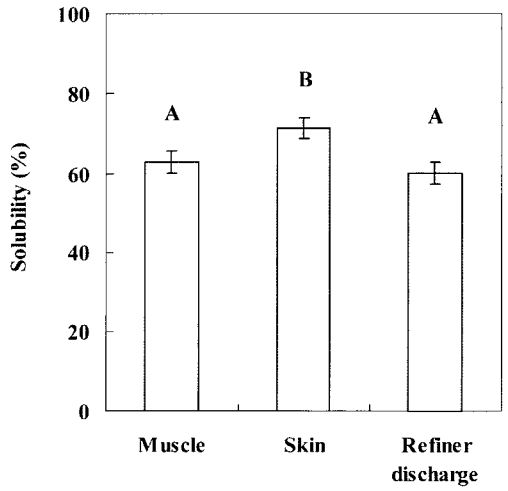

Fig. 3. Solubility of collagen from various solid by-products and muscle of Alaska pollock. Different letters on the bars indicate a significant difference at $p<0.05$.

Table 3. Amino acid composition of collagens from various solid by-products and muscle of Alaska pollock

(residues/1,000 residues)

Amino acid	Collagen source		
	Muscle	Skin	Refiner discharge
Aspartic acid	35.0	37.9	32.0
Hydroxyproline	66.6	60.6	68.8
Threonine	31.2	31.7	32.3
Serine	62.2	59.2	61.8
Glutamic acid	72.0	69.6	77.0
Proline	102.4	100.1	101.9
Glycine	325.5	323.6	328.7
Alanine	118.0	116.2	113.4
Cystein	ND[1)]	ND	ND
Valine	22.4	24.1	22.7
Methionine	15.1	15.1	14.8
Isoleucine	11.8	13.8	12.4
Leucine	22.3	25.3	22.5
Tyrosine	1.5	2.2	1.6
Phenylalanine	15.4	15.9	15.6
Hydroxylysine	9.8	8.8	9.4
Lysine	28.2	31.5	27.3
Histidine	7.8	9.1	7.3
Arginine	52.8	55.3	50.5
Total	1,000	1,000	1,000

[1)]Not detected.

of fish, fish species, and/or differenec methodology (26). The acid-solubilities of collagens from solid by-products and whole muscle of Alaska pollock were similar to those of the fish collagens from other fishes, such as brown rockfish (64.8%) (27) and chilipepper rockfish (63.4%) (27), while was higher than those of collagens from land animal collagen (54.6%) (27). These fact was probably because of the difference in imino acid content between collagens from fish and land animal (28). It was well-known that imino acid is involved inter-chain hydrogen bonding, which stabilizes the triple helical structure of collagen (29, 30).

Amino acid composition Amino acid compositions of collagens from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Table 3. Glycine ranged from 324 to 329 residues/1,000 residues and it was the most abundant amino acid present in all collagen samples. Regardless of the kinds of collagen, alanine (113-118 residues/1,000 residues) and proline (100-103 residues/1,000 residues) compositions were also rich. However, cysteine (not detected), methionine (14.8-15.1 residues/1,000 residues) isoleucine (11.8-13.8 residues/1,000 residues), tyrosine (1.5-2.2 residues/1,000 residues), phenylalanine (15.4-15.9 residues/1,000 residues), hydroxylysine

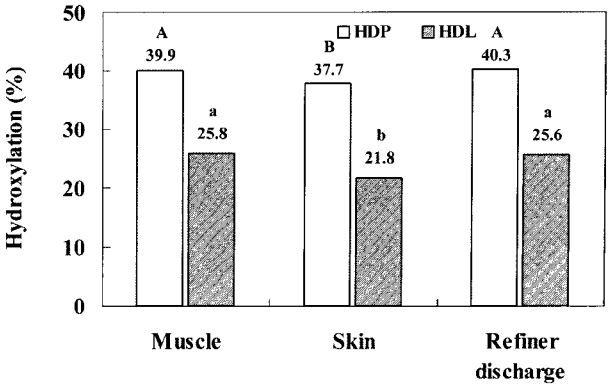

Fig. 4. Hydroxylation ratio of proline (HDP) and lysine (HDL) in acid-soluble collagen from various solid by-products and muscle of Alaska pollock. Different letters on the bars in the same experiment item indicate a significant difference at $p<0.05$.

(8.8-9.8 residues/1,000 residues), and histidine (7.3-9.1 residues/1,000 residues) showed a significantly low concentrations when compared to the other amino acids. Similar pattern of amino acid composition of collagen samples was also found in skin and muscle of various marine animals, such as squid mantle (29), hake (*M. merluccis* L.) (8), trout (8), carp (17), eel (17), common mackerel (17), saury (17), chum salmon (17), filefish (31), lobster (17), top shell (17), sea cucumber (32), Pacific whiting (1), and Alaska pollock (33).

Hydroxylation ratio of proline and lysine Hydroxylation ratios of proline and lysine generally affect the functional properties of gelatin that can be derived from collagen as well as the thermal stability of collagen (30). Hydroxylation ratios of proline (HDP) and lysine (HDL) in acid-soluble collagen from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Fig. 4. The highest hydroxylation ratio of proline was obtained from collagen from refiner discharge (40.3%), followed by collagen from whole muscle (39.9%), and collagen from skin (37.7%). However, there was no significant difference ($p>0.05$) in the hydroxylation ratio of proline between collagens from refiner discharge and whole muscle. This might be due to refiner discharge is connective tissues separated from washed mince. There was also no significant difference ($p>0.05$) in hydroxylation ratio of lysine between collagens from refiner discharge (25.6%) and whole muscle (25.8 %). On the other hand, hydroxylation ratio of lysine of 2 collagens above were high when compared to that of collagen from skin (21.8%). Ando *et al.* (29) and Montero *et al.* (8) reported that hydroxyproline plays a role in stabilizing the triple helix, whereas hydroxylysine contributes to the formation and stabilization of cross-links of nonhydrolyzable bonds. Kimura *et al.* (17) and Zhu and Kimura (18) also reported that hydroxylation ratio of proline was higher in muscle collagen than in skin collagen. Based on our results and the literature, the thermal stability of collagen from refiner discharge is likely high when compared to that of collagen from skin. The hydroxylation ratio of proline in collagen from refiner discharge was 40.3%, which was similar to those of

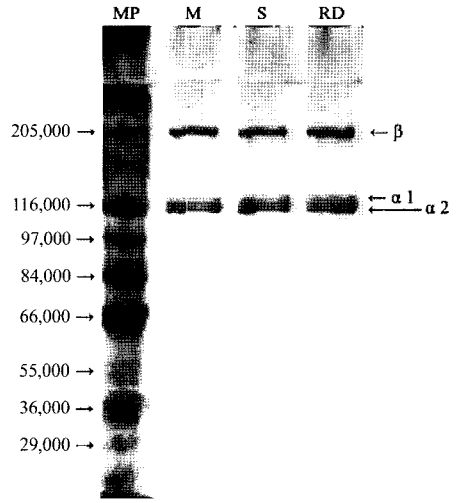

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic pattern of acid-soluble collagens from various solid by-products and muscle of Alaska pollock. MP, marker protein; M, acid-soluble collagen from muscle; S, acid-soluble collagen from skin; RD, acid-soluble collagen from refiner discharge.

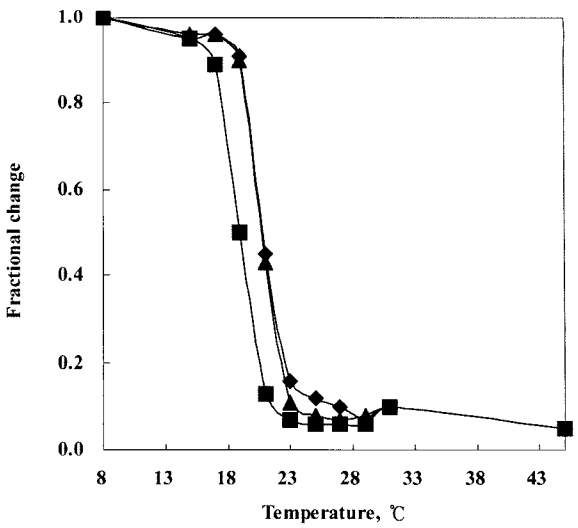

Fig. 6. Fractional change in specific viscosity of acid-soluble collagen from various solid by-products and muscle of Alaska pollock. The denaturation temperature was measured by viscosity in 0.1 M acetic acid of 0.03% collagen solution.

collagens from muscle of common horse mackerel (38%), yellow sea bream (40%), and tiger puffer (39%) (34). However, it was much lower than those from shark, carp (34), land animals, and other higher vertebrates (24).

SDS-PAGE pattern Sodium dodecyl sulfate-polyacrlamide gel electrophoretic (SDS-PAGE) patterns of acid-soluble collagens from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Fig. 5. According to SDS-PAGE pattern, 3 chains, 2 α bands (α1, upper; α2, lower) with their molecular weight at about 100 kDa and their β cross-linked components, with a molecular weight of 200 kDa, were detected in all collagens from skin, refiner discharge, and whole muscle. As a whole, our electrophoretic patterns of collagens from skin and refiner discharge were almost identifical to those of the corresponding calf skin type I collagen in mobility of chains (data not shown) and similar to those obtained with collagens from the skin and muscle of other species, such as hake (*Merluccius hubbsi*) (9), (*M. merluccis* L.) (8), Pacific whiting (1), trout (8), and Alaska pollock (33). There was no difference in the relative mobility of α1 and α2 chains between acid-soluble collagens from skin and refiner discharge. Based on our results and the literature, the collagens obtained in this experiment were found to be free of noncollagenous proteins.

Thermal denaturation temperature Investigations on the thermal denaturation temperatures (TDT) of the native collagen molecule deal with processes associated with the collagen-gelatin transition (28). The fractional change in specific viscosity of acid-soluble collagen from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Fig. 6. There was no difference in TDT between collagens from refner discharge (20.7°C) and whole muscle (20.6°C). No difference in TDT between whole muscle and refiner discharge was probably because refiner discharge is connective tissue separated from washed mince. The TDT of collagen from Alaska pollock skin was 19.0°C, which was slightly low when compared to that of collagen from refiner discharge (20.7°C). The result was probably because the hydroxylation ratio of proline of refiner discharge collagen (40.3%) was 2.5% higher than that of skin collagen (37.7%). However, the TDT of collagen from refiner discharge was much lower than those of collagens from shark (35), carp (36), and land animal skins (37). These results suggested that gelatin from refine discharge of Alaska pollock cannot be used in products demanded heat-reversible properties because gelatin gel melts at around room temperature. For effective use of collagens from surimi by-products, such as skin and refiner discharge, of Alaska pollock as food or other industrial ingredients, therefore, their thermal stability must be improved.

Water and oil absorption capacities Water (WAC) and oil (OAC) absorption capacities of acid-soluble collagen from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Fig. 7. WACs of collagens from whole muscle and refiner discharge of Alaska pollock were 8.3 and 8.1 mL/g, respectively. No significant difference ($p>0.05$) in WAC between collagens from whole muscle and refiner discharge of Alaska pollock was probably because refiner discharge is connective tissues separated from washed mince. WACs of collagens from whole muscle and refiner discharge of Alaska pollock were, however, high when compared to that of collagen from skin of Alaska pollock (4.6 mL/g). WAC of collagen from refiner discharge was also high when compared to those of vegetable proteins, such as soybean protein isolates (3-8 mL/g) (38), protein from alfalfa leaf (20) (1.85-3.58 mL/g), and soybean protein (1.67-5.93 mL/g) (39). This difference may be due to the high swelling ability of collagen (40) along with the difference of size, shape, hydrophilic-hydrophobic balance of amino acids in

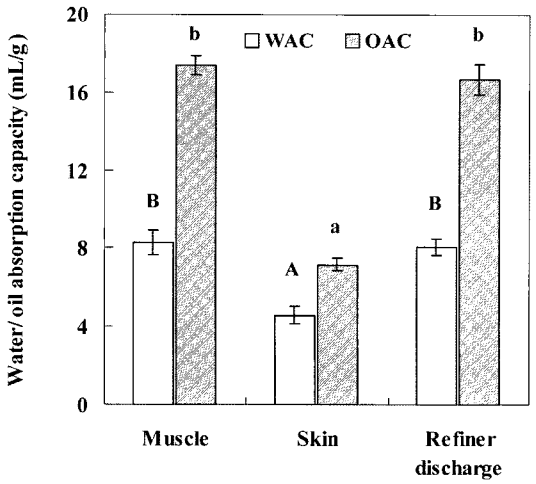

Fig. 7. Water absorption capacity (WAC) and oil absorption capacity (OAC) of acid-soluble collagen from various solid by-products and muscle of Alaska pollock. Different letters on the bars in the same experiment item indicate a significant difference at $p<0.05$.

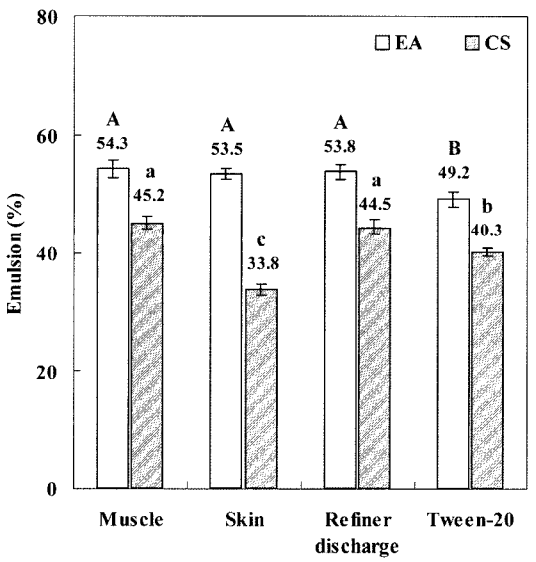

Fig. 8. Emulsion activity (EA) and cooking stability (CS) of acid-soluble collagen from various solid by-products and muscle of Alaska pollock. Different letters on the bars in the same experiment indicate a significant difference at $p<0.05$.

the protein molecule and the physicochemical environment, such as pH, ionic strength, and temperature (39).

OAC of acid-soluble collagen from refiner discharge (16.7 mL/g) was no significant difference ($p<0.05$) when compared to that of collagen from whole muscle, while was high when compared those of soybean protein isolates (2-10 mL/g) (38), protein from alfalfa leaf (1.75-4.30 mL/g) (20), soybean protein concentrates (1.00-4.12 mL/g) (39), and salmon hydrolysates (2.86-7.07 mL/g) (41) as well as that of collagen from skin (7.2 mL/g).

Emulsion activity and cooking stability Emulsion activity (EA) and cooking stability (CS) of acid-soluble collagens from skin, refiner discharge, and whole muscle of Alaska pollock are shown in Fig. 8. EA of acid-soluble collagen from refiner discharge showed 53.8%, which was high when compared to that of commercial emulsifier (Tween-20, 49.2%), and was similar to those of collagens from whole muscle (54.3%) and skin (53.5%). EA of collagen from refiner discharge is primarily determined by the orientation at the interface between the 2 phases where a monomolecular film is formed around the colloidal particles (42).

Regardless of kinds of acid-soluble collagens used in this experiment, CS was high compared to EA. EA of acid-soluble collagen from refiner discharge showed 44.5 %, which was high when compared to that of commercial emulsifier (Tween-20, 40.3%) as well as that of collagen from skin (33.8%). EA of acid-soluble collagen from refiner discharge was, however, no significant difference ($p<0.05$) when compared to that of collagen from whole muscle (45.2%). Kim *et al.* (43) reported that emulsifying properties of gelatin from cod bone were similar to those of a commercial emulsifier, such as Tween-60 and Tween-80.

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