

Physico-chemical and functional properties of gelatin from Surimi processing byproducts (Refiner discharge)

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Surimi processing industries generate large quantities of waste of which refiner discharge is of one kind. Gelatin extraction from such waste has not been attempted widely. Gelatin was extracted from pink perch (*Nemipterus japonicus*) surimi refiner discharge and its physico-chemical and functional properties were studied. The gelatin yield was 15.86 % on dry weight basis. The bloom strength, viscosity and melting point of the refiner discharge gelatin were recorded as 147.9 g, 7.44 cP and 25.5°C, respectively. The refiner discharge gelatin is rich in glycine followed by glutamic acid, proline and alanine. The gelatin has high intensity of β - and α -chains as the major components. Emulsifying capacity and emulsion stability of gelatin were 50.77% and 50.44%, respectively; indicating the extracted gelatin from refiner discharge had strong emulsion stability. The refiner discharge gelatin was also found to have good foam expansion of 38.45 %. It can be concluded from the present study that fish surimi refiner discharge can be a potential source for gelatin production. Byproducts like refiner discharge have added advantage of avoiding the segregation problem of solid waste into skins, scale and bones before the extraction of gelatin.

Key Words : Refiner discharge, Gelatin, Physico-chemical, Functional, Waste

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INTRODUCTION

Gelatin is a soluble proteins obtained from partial hydrolysis of collagen (Shakila *et al.*, 2012). There are two main types of gelatin based on the isoionic point, Type A (7 to 9) and Type B (4 to 5) resulting from different pretreatments (Baziwane and He, 2003). Gelatin is used

for the production of jelly, confectionaries, dairy product, meat products and canned foods due to its melt-in-the-mouth sensation. Gelatin also find used in pharmaceutical industry in encapsulation, tablet coating, emulsions and cosmetics (Shakila *et al.*, 2012). Fish gelatins are of interest in the food processing industry after outbreak of Bovine spongiform encephalopathy and also due to religious concerns of gelatin derived from bovine and porcine (Sadowska *et al.*, 2003). This has led to intensive research on aquatic resources based-gelatin to be an alternative to mammal-derived gelatin. Several authors have reported extraction of gelatin from several fishes such as skipjack tuna, dog shark, and rohu, red snapper and grouper (Shyni *et al.*, 2014 and Shakila *et al.*, 2012). Surimi processing involves processing whole or gutted

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fish into mince, repeated washing of the mince and refining. Refining is a screening mechanism, where the remaining scale, connective tissues and bones are separated from the mince. The solid by-products from surimi industries does not find used as a human food. Most surimi solid by-products are conventionally used to produce fish meal and fertilizer or are directly discharged into estuaries, resulting in eutrophication (Ciarlo *et al.*, 1997). Refinishing step in surimi manufacturing produces 15-22 % refiner discharge which constitutes 4-8 % connective tissue of the whole fish (Wendel, 1999). Such waste can form an ideal raw material for gelatin production. There is a little information regarding the utilization for surimi by-product like refiner discharge for the production of gelatin. Our objective was to study the physico-chemical characteristics and functional properties of gelatin extracted from pink perch (*Nemipterus japonicus*) surimi refiner discharge.

METHODOLOGY

Materials and chemicals :

Pink perch surimi refiner discharge was collected from a commercial surimi processing plant (Ulka Seafoods Pvt Ltd., Maharashtra, India) and transported in ice to the laboratory where it was kept frozen at -30°C until used for gelatin extraction. Sodium Hydroxide (NaOH), Ethylenediaminetetraacetic acid (EDTA), Hydrochloric acid (HCl) and glycerol were procured from SRL, Mumbai. Sodium Dodecyl Sulphate (SDS), Coomassie blue, Ammoniumpersulphate (APS) and Tetramethylenediamine (TEMED) were obtained from MERCK, Germany.

Gelatin extraction :

Gelatin was extracted from surimi wastes following the methods described by Wang and Regenstein, 2009 with slight modifications. Thawed refiner discharge was soaked in 0.20 M NaOH with a waste/solution ratio of 1:10 (w/v) for 2 h at room temperature and the alkaline solution was changed every 1 h interval in order to remove non collagenous proteins and pigments. The treated waste was then washed with potable water until neutral pH of wash water was obtained. The refiner discharge was then soaked in 0.20 M EDTA with a waste/solution ratio of 1:10 (w/v) for 2 h to swell the collagenous material. Again the acid pre-treated skin was washed thoroughly with potable water until wash water became neutral. The

swollen refiner discharge was soaked in distilled water with a waste/water ratio of 1:3 (w/v) in a temperature-controlled water bath at 70 °C for 3 h with an occasional stirring. The filtrate was concentrated in flash evaporator (BUCHI, India) at 45°C until the volume become half, followed by drying the solution in vacuum dryer (Heraeus vacuotherm, Germany) for 12 h. The yield of gelatin was calculated based on the weight of dry gelatin in comparison with that of fresh waste weight.

Chemical composition :

Proximate composition such as moisture content, crude protein, fat and ash were estimated by standard AOAC, 2005 methods. The pH of 1% (w/w) gelatin solution was measured using a digital pH meter (pH tester, HANNA, USA).

Bloom strength :

Bloom strength was measured according to the method described by Gelatin Manufacturers Institute of America (GMIA, 1986).

Viscosity :

The viscosity of the gelatin (6.67% concentration at 60 °C) was measured using a Brookfield digital viscometer (model DV-E, Brookfield Engineering, Middleboro, MA, USA) equipped with a No. 1 spindle at 30 ± 0.5 °C. The measured values were obtained directly in centipoises (cP) from the instrument.

Melting point :

Determination of melting point was based on the JIS K6503 1996 method.

Setting point and Setting time :

Setting point (SP) and Setting time (ST) of gelatin was assessed by following Muyonga *et al.* (2004) method.

Water holding capacity, fat binding capacity, foam expansion (FE) and foam stability (FS) :

The functional properties of the gelatin such as water holding capacity (WHC), Fat binding capacity (FBC), FE and FS were determined using the Balti *et al.* (2011) method.

Amino acid analysis :

100 mg of defatted gelatin was hydrolyzed at 110°C

for 24 h in a test tube with 10 ml of 6 N HCl. After the hydrolysis the filtrate was flash evaporated to dryness to remove traces of HCl. The residue was then dissolved and made upto 10 ml with 0.05 M HCl and then filtered through a membrane filter (Thermo Scientific, MK, England) with a nominal pore size of 0.45 μm . The sample (10 μl) was injected into the amino acid analyzer (HPLC–LC 10 AS, Hitachi L-2130 Elite La Chrome, Japan). The identification of amino acids in the sample is done using D-2000 Chromatography Data Station Software.

Determination of molecular weight:

SDS-PAGE of the extracted gelatin was done according to the method of Laemmli 1970. Gelatin was dissolved in 5% SDS and heated at 80°C for 30 min followed by centrifugation (HERAEUS multifuge X1R, Thermo Scientific,) at 3500 x g for 5 min. The protein was diluted to 1:1 (v/v) with sample buffer (0.5 M Tris HCl, Ph6.8 containing 10 % SDS, 10 % glycerol and β -mercaptoethanol) in microcentrifuge tube and boiled for 3 min at 100°C. 10 μl of the solution was loaded onto the polyacrylamide gels (7.5 % separating gel and 4 % stacking gel) and electrophoresis was done at a constant current of 15 mA/gel using a Mini Protein II unit (Bio-Rad Laboratories, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid for 30 min and destained with 30% (v/v) methanol and 10% (v/v) acetic acid for 2 h. Wide range molecular weight protein marker (Sigma Chemical Co., St. Louis, MO, USA) with a molecular weight range of 6500-200,000 Da was used to estimate the molecular weight of proteins.

Statistical analysis :

One way analysis of variance (ANOVA) was used to determine descriptive statistics in IBM SPSS (20).

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Chemical composition :

The yield of the gelatin from pink perch refiner discharge was 15.86 % on the basis of dry weight. The present yield is higher than the yield of gelatin extracted from tuna skin, however slight lower than gelatin from shark skin and rohu skin (Shyni *et al.*, 2014). The yield of the gelatin generally depends on the composition of the raw material and sufficient denaturation of soluble collagen during the extraction etc. The proximate compositions of the refiner discharge and refiner discharge gelatin are given in Table 1. The moisture content of the refiner discharge gelatin is lower than the required moisture content of 15 % for the edible gelatin. The gelatin was also found to have low ash content, suggesting EDTA treatment could chelate minerals from the refiner discharge. Lower ash content contributes to high quality of gelatin.

Physical characteristics of the refiner discharge gelatin :

All the determined physical properties of the extracted gelatins were tabulated in Table 2. The gelatin extracted from pink perch refiner discharge belongs to Type-B gelatin as it has low pH value of 3.69. Wide variations in the pH of gelatin extracted from fishes were reported such as fresh water carps (4.01–4.88) and bigeye snapper of 6.44 (Ninan *et al.*, 2011 and Binsi *et al.*, 2009). This was mainly because of the different pretreatments employed during the extraction involving both alkaline and acid treatments.

Bloom strength is the most significant physical properties of gelatin. The application of gelatin is determined by the range of gel strength values. The result value is low when compared to that of gelatin from the skins of warm water fish such as grass carp (267 g) and tilapia (328 g) (Kasankala *et al.*, 2007 and Songchotikunpan *et al.*, 2008). Bloom value of fish gelatin ranges from as low as zero to 426 g, compared to 200–300 g for bovine or porcine gelatin (Karim and Bhat, 2009). But the present bloom value of refiner discharge gelatin result is higher than the values obtained from cold-water fish species such as salmon (108 g) and cod 70–

Table 1 : Proximate composition of raw material and extracted gelatin powder

Source	Moisture	Protein	Crude lipid	Ash
Surimi refiner discharge	73.95 \pm 0.65	13.67 \pm 2.49	2.88 \pm 0.02	10.61 \pm 2.73
Extracted gelatin	7.09 \pm 0.20	92.84 \pm 1.47	0.39 \pm 0.03	0.84 \pm 0.05

Values are given as mean \pm standard deviation of triplicate

90 g (22, 23), while that for rohu it was 124 g (Shyni *et al.*, 2014). Bloom strength not only depends on average molecular weight of gelatin but also is dependent on other factors such as the chemical treatment of raw collagen material, type and concentration of the gelatin and the time/temperature history of the sample. Bloom strength also depends on the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin (Arnesen and Gildberg, 2007). Gelatin is compatible with a wide variety of foods and ingredients. Gelatin of different bloom strength from the same source

can be produced by merely varying the extraction method (Jamilah *et al.*, 2011). Thus, gelatin extracted from refiner discharge can find application in products like soft gelatin capsule, ice-cream etc.

Viscosity is considered as the second most important rheological properties of gelatin solutions. The viscosity of extracted gelatin was 7.44 Cp. pH are known to influence the viscosity and minimum viscosity for gelatin has been reported in the pH range of 6–8. Jamilah *et al.*, 2011 reported the viscosity values of 3.2 cP and 7.12 cP for red and black tilapia, respectively. The viscosity of grouper bone gelatin and red snapper were reported to be 18.5 cP and 15.30 cP, respectively (Shakila *et al.*, 2012).

The melting point of extracted gelatin was found to

Table 2 : Physico-chemical properties of extracted gelatin from pink perch surimi refiner discharge

Physical properties	Extracted gelatin
pH	3.69 ± 0.11
Gel strength (g)	147.97 ± 1.78
Viscosity (cP)	7.44 ± 0.14
Melting point (°C)	25.5 ± 0.50
Setting point (°C)	18.5 ± 0.50
Setting time (s)	120.66 ± 2.08

Values are given as mean ± standard deviation of triplicate

Table 3 : Amino acids composition of extracted gelatin (g / 100 g sample)

Amino acids	Content (g / 100 g gelatin)
Asparagine	3.27
Alanine	5.48
Arginine	5.26
Glutamine	9.15
Glycine	26.12
Histidine	2.05
Isoleucine	0.54
Leucine	1.61
Lysine	1.90
Methionine	1.03
Phenylalanine	2.38
Proline	7.55
Hydroxyproline	10.45
Serine	2.89
Threonine	1.45
Tyrosine	5.23
Valine	1.43

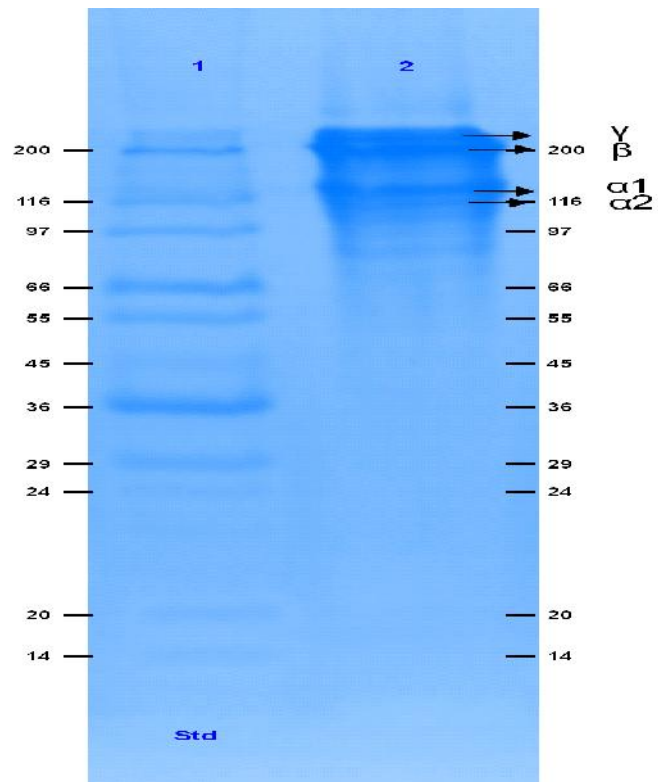


Fig. 1 : SDS-PAGE pattern of extracted gelatin (lane 1: standard; lane 2: sample)

Table 4 : Functional properties of pink perch surimi refiner discharge gelatin

Foam expansion (%)	Foam stability (%)		Emulsifying capacity (%)	Emulsifying stability (%)	Emulsion activity index (m ² /g)	WHC (ml/g)	FBC (ml/g)
	30 min	60 min					
38.45 ± 1.63	24.80 ± 1.67	20.16 ± 0.76	50.77 ± 0.58	50.44 ± 0.66	55.53 ± 0.44	2.20 ± 0.26	3.06 ± 0.15

Values are given as mean ± standard deviation of triplicate.

WHC=Water holding capacity.

FBC=Fat binding capacity.

be higher than the results of Unicorn leatherjacket skin (22.61° C) (Hanjabam *et al.*, 2015) and other gelatin extracted from cold water fishes (Gomez-Guillen *et al.*, 2002). The melting point of gelatin is the point at which the gelatin gel starts melting when the temperature increases above a certain point. Typical melting points for fish gelatins range from 11 to 28° C. Viscosity is partially controlled by molecular weight and molecular size distribution (Karim and Bhat, 2009). Also fish gelatin with low bloom value and low melting point can accelerate the flavour release than high bloom value and higher melting point gelatin.

Gel setting point is defined by the temperature at which gelling process begins by transforming from random coil to triple helical structure of gelatin (Haug *et al.*, 2004). Gelatin solution from refiner discharge gelled at 18.50° C. Gelatins from skins of salted and marinated herrings formed a gel at 5 °C and from cod skins at about 5.5° C. According to Gomez- Guillen *et al.* (2002) gelling of cod gelatin occurred at 12–13° C. Gelling temperature depends on the imino acid composition, ionic strength and pH of the gelatin. The imino acids were found to stabilize the ordered conformation when gelatin forms the gel network during gelling and with the increase in ionic strength of >0.5 mol/L decreased gelling temperature, which was probably due to the reduced electrostatic interaction preventing attractive ionic inter-chain bridging and gelation of fish gelatin (Muyonga *et al.*, 2004 and Haug *et al.*, 2004).

Gelling time required for extracted gelatin was 120.66 s. Similarly, Ninan *et al.*, 2011 also observed higher gelling times in rohu (106 s) and common carp (103 s) skin gelatins.

Functional properties of the refiner discharge gelatin :

All the determined functional properties of the extracted gelatin were tabulated in Table 4. Water holding and fat binding capacities (WHC and FBC) are important functional properties. They are related to the ability of protein to imbibe water, oil and retain it against a gravitational force within protein matrix. The WHC and FBC of extracted gelatin were found to be 2.20 ml/g and 3.06 ml/g, respectively. High WHC gelatin could be a suitable material for coating material to reduced drip loss in frozen fish or meat products. Higher fat binding capacity is related to the degree of exposure of the

hydrophobic residues and the high amount of tyrosine in the gelatin (Ninan *et al.*, 2011).

Emulsifiers are surface active materials that adsorb to interfaces and facilitate the production of small droplets by lowering the interfacial tension during homogenization. Proteins are widely used as emulsifiers in food products because of their ability to improve the stability of oil-in-water emulsions. Gelatin is surface-active and is capable of acting as an emulsifier in oil-in-water emulsions (Aewsin *et al.*, 2009). The characteristics used to describe emulsifying properties of protein are Emulsin capacity (EC), Emulsion stability (ES) and Emulsion activity index (EAI). EC and ES of gelatin from refiner discharge were 50.77% and 50.44%, respectively indicating the extracted gelatin had strong emulsion stability. The EAI was 55.53 (m²/g). This EAI is higher than that of bovine and fish gelatin reported by Aewsin *et al.* (2009). Surh *et al.* (2006) found that the oil-in-water emulsion prepared with high molecular weight fish gelatin (~120 kDa) was more stable than that prepared with low molecular weight fish gelatin (~50 kDa). Also EC and ES depend on the properties of protein, its concentration, pH, ionic strength and viscosity.

The foaming ability of proteins is related to their film-forming ability at the air–water interface. The extracted gelatin was found to have good foam expansion of 38.45 %. The foaming stability of gelatin was found to be higher at both 30 and 60 min. A protein must be capable of migrating rapidly to the air-water interface; unfolding and rearranging at the interface to express good foaming properties (Aewsin *et al.*, 2009).

Amino acid composition :

The amino acids profile of extracted gelatin from pink perch refiner discharge is given in Table 3. The extracted gelatin is rich in glycine followed by glutamic acid, proline and alanine. During acid extraction of gelatin some of the side-chain amides like glutamine and asparagine get converted to their respective acidic forms (Jamilah *et al.*, 2011). In general glycine is the most predominant amino acid in any gelatin as glycine occur every third position in the chain and represents nearly one third of total amino acids residues. However, glycine content in the extracted gelatin was slightly lowered than shark skin, rohu skin and tuna skin gelatin (Shyni *et al.*, 2014). Proline and hydroxyproline are important for rigidity and stability of the triple helix of collagen which affects

the functionality of the gelatin (Gomez-Guillen *et al.*, 2002).

Molecular weight distribution :

The gelatin extracted was analyzed using SDS-PAGE (Fig. 1). The gelatin has high intensity of β - and α -chains (α_1 and α_2) as the major components though a faint γ - chain is also visible. Bands of low peptides were not noticeable. Some authors have observed lower MW fractions of gelatin, particularly due to cleavages of protein chains at high temperature extraction process (Muyong *et al.*, 2004 and Shyni *et al.*, 2014). According to Gomez-Guillen *et al.* (2002) damage or partial losses of α chain occurring due to the heat extraction and gel preparation were responsible for the lower MW fractions.

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