World Journal of Fish and Marine Sciences 3 (1): 10-15, 2011
ISSN 2078-4589

Extraction and Evaluation of Gelatin from Silver Carp Waste

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Abstract: Gelatin was extracted from skins and fins of silver carp fish named fitofague by acidic and alkaline methods with the yield 7.5% in acidic and 6.5% in alkaline method. Quality factors of gelatin such as nutritional components, gel strength, viscosity and melting temperature in two type's gelatin were determined and compared with each other. Rheological properties of alkaline gelatin like gel strength and viscosity are higher than acidic gelatin. Gelatin derived from silver carp wastes have higher protein content (86-88%) and lower gel temperature (7-10°C) than mammalian gelatin. In conclusion gelatin extracted from fitofague wastes has good quality and can be used in the food industries.

Key words: Gelatin % Fish Offal % Extraction % Functional Properties

INTRODUCTION

The amount of gelatin used in the world-wide food industry is annually increasing. The worldwide production of gelatin was about 326000 metric tons, of which 46% from pigskin, 29.4% from bovine hides, 23.1% from bones and 1.5% from other parts [1]. Gelatin is a substantially pure protein food ingredient, obtained by heat denaturation of collagen that may be considered a highly digestible dietary food ideal as a complement in certain types of diet.

In the food industry, gelatin is one of the water soluble polymers that can be used as an ingredient to improve the elasticity, consistency and stability of foods. There are two main types of gelatin. Type A, with isoionic point of seven to nine, is derived using exclusively acid pretreatment. Type B, with isoionic point of four to five, is the result of an alkaline pretreatment. The quality of gelatin depends to a large extent on its rheological properties mainly gel strength and viscosity, but other characteristics particularly transparency, absence of color and flavor and easy dissolution are also important [2].

Gelatin is one of the most consumed colloids in food, pharmaceutical and other industries that produced in four grades pharmaceutical, edible, photography and industrial. It is used for various applications in the food industries such as jelly production confectionary, edible films, encapsulation, fruit juice clarification, dairy processing, soups and other applications. The issue of gelatin replacement has existed for many years for the vegetarian, halal and kosher markets, but has gained increased interest in the last decade. During recent years, the neurological disease called Bovine Spongiform Encephalopathy (BSE) have been seen in some countries that some of them were exporter of cow meat and meat products. Despite due to harsh treatment in gelatin production, probability of prion survive is very low, but consumers still worried about it. In the other hand, in Islam and Jewish, consumption of pork meat and its related products such as gelatin is forbidden [3].

Therefore, it is necessary find new sources of gelatin like fish wastes which produced in large amounts during fillet and canning processes. The waste from fish processing after filleting can account for as much as 75% of the total catch weight. About 30% of such waste consists of skin and bone with high collagen content [1]. The main difference between fish and mammalian gelatin is the content of the imino acids proline and hydroxyproline, which stabilizes the ordered conformation when gelatin forms a gel network. The lower content of proline and hydroxyproline probably gives fish gelatin its low gel modulus, gelling and melting temperature [4].

There are extensive researches on different aspects of gelatin production, processing and properties. These researches focus on find the optimum parameters of gelatin extraction from various sources, physiochemical, rheological and nutritional properties of extracted gelatin and investigation for new gelatin derivatives or modified gelatin like coldwater soluble gelatin, hydrolyzed gelatin, esterified gelatin, encapsulation and edible film based

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gelatin. Kazemi visari [5] investigated gelatin extraction from various sources like hen's skin and foot, sheep and cow bone.Some researches accomplished on optimization of gelatin extraction from fish processing [6-9]. Fish gelatin quality and nutritional properties like physiochemical characteristics, rheological properties and amino acid analysis are subject of many investigations [1, 10- 12]. Choi and Regenstein [13] found fish gelatin to have similar physical and chemical properties compared to porcine gelatin and to be rated superior in a blind sensory test. They also established that the lower melting point of coldwater fish gelatin enhances flavor release, fruit aroma and melt rate in water gel desserts. Recently, edible film producing ability of marine based gelatin with different additives and antioxidants such as plant extracts are studied by some researchers.

The main problem of marine gelatins is their inferior rheological properties, which may limit their application field. To overcome or minimize some of the problems associated with inferior properties of fish gelatin, three different approaches have been attempted: enzymatic cross-linking of gelatin using enzymes such as transglutaminase or tyrosinase, mixed gelling systems of fish gelatin and suitable plant hydrocolloids which may give higher gel strength, gelling and melting temperature and manipulating the characteristics of gelatin by the addition of solutes, such as salts [4].

The silver carp fish also known as Fitofague is commonly cultured in many parts of the country and world. Fitofague is cultured in ponds and available in Iranian fresh water fish market almost all year round and it has potential processed into fillet and cans. The objective of this investigation was to study some physiochemical and rheological properties of gelatin extracted from skin and fin of silver carp fish (fitofague) with acidic and alkaline methods.

MATERIALS AND METHODS

Silver carp fish purchased from cultivating ponds located at Tehran suburb. The samples were placed in ice and transported to laboratory of university within 2 h. Upon arrival, Skin and fin were cleaned by washing with tap water, drained and kept at - 20°C until used for extraction.

Gelatin extraction by acid and alkaline methods based on put samples in water bath at 50, 60 and 70°C.These are optimum temperatures for extraction because lower temperature result lower yield and higher temperature leads to lower gelatin quality [14].

Acidic Extraction Method (Type A): 1100g fitofague's skin and fin unfrozen at room temperature, cut to 10-30 cm pieces and put to water bath includes 3800ml water along 3.6 ml sodium hypochlorite or hydrogen peroxide (for microbial growth prevention) for 20 minutes. Subsequently, rinsing with 3 liter clean water for 15 minutes then put to acid bath with addition 2.5 liter water and 12.6 ml glacial acetic acid at pH3.5 and remain for 5 hours. During this time, the undesirable components extracted and texture was soft and ready for gelatin extraction. Organic acids like lactic and acetic acids are better than sulphuric and chloridric acids due to stronger acid cause denatured collagen proteins resulted lower quality gelatin, but possible gave higher yield. According to literature, 3-20 liter per tone fish skin recommended, in this research used averaged value 11.5 ml per tone fish skin. After elapsed 5 hours mixed fin and skin were rinsed by clean water in two 30 minutes steps until gave pH 4.5-5.Then samples poured to beaker includes 3400 ml water at 90°C for four hours then transferred to water bath at 50°C. During this period temperature held constant at 50°C and pH at range 4.5-5. Process continued four hours for completed extraction. The extracted gelatin solution is bleached with charcoal then filtered through diatomaceous earth (3g/l) to remove suspended insolubles such as lipids or unhydrolyzed collagen fibers. The gelatin is then further purified through ion exchange or ultra filtration columns, which remove inorganic salts and removed any off-flavor due to amine and it's derivatives [14]. Gelatin solution concentrated through rotary vacuum evaporator to 5% concentration then dehydrated in convective oven at 50-60°C until gelatin became dry and thin layers.

Alkaline Extraction Method (Gelatin Type B): 1100g Fitofague's skins and fins were unfrozen at room temperature then cut to 10-30cm pieces and further soaked in calcium hydroxide solution at 15-20°C.Minimum required calcium hydroxide for fatty fish like silver carp is 50g/l that prevent from putrification during test. In several trials, the optimum concentration of calcium hydroxide was selected as 75g/l per each kilogram fitofague skins and fins and optimum process time was selected as 2-4 weeks. Therefore 82.5g calcium hydroxide solved in 1100ml water for 4 weeks until pH sample was greater than 12. Longer pretreatment time or higher concentration of Ca(OH)2 could significantly reduced gelatin quality. After passed 4 weeks, material was rinsed until pH =10 and held constant during extraction process. Afterwards, gelatin

solution was filtered and mixed with choloridric acid 5% (w/w) while pH5.However, for pH normalization can used from cation ion exchange resin (strong acid). remained steps was done like acid method.

Physiochemical and Rheological Tests: After gelatin production by two above mentioned methods, quality factors were determined according to national and international standards.

Moisture Content: Moisture content was determined by gravimetric method.[15].

Ash: Ash content determination as described by national standards [15].

Protein: Protein content determination using the Kjeldahl method. Conversion factors were 5.51 and 5.46 for alkaline and acidic methods, respectively [16].

pH: pH was determined by pH meter (HORIBA,F12,Japan) [15].

Amino Acid Composition: Amino acid composition of the samples were determined by using of the high performance liquid chromatography (HPLC) method [17].

Setting Point and Setting Time: Setting point & time were determined according to *Muyonga et al*. [18]. For setting point determination first 10% (w/v) gelatin solution was prepared in warm water bath then 30 ml transfer to test tube (12mm × 75mm) then placed to water bath at 40°C. Then cooling water bath by slowly addition of cold water at 2C for time intervals 15s. Thermometer put to the solution and out each 15s until any drop doesn't drip; this temperature was recorded as gelatin setting point.

Melting Point and Melting Time: Melting point & time were determined according to *Muyonga et al*.[18]. 10% (w/v) gelatin solution was prepared like past section, put to the refrigerator at 7°C for 16-18 hours then transfer to the water bath at 10°C with gradually addition of warm water (45°C) and melting temperature and time was recorded.

Color Determination: Color of gelatin samples were measured by putting them on white background and compared with each other. Gelatin color must be pale yellow to amber.

Gel Strength: The significant characteristic of commercial gelatin is gel consistency that determined by gel strength. It was determined according to BSI [19]. 7.5g gelatin was mixed with 105ml distilled water then poured to bottle. This mixture standup at room temperature for 30 minutes until the solution was swollen. Afterwards it was heated in the water bath at 42°C for 30min until gelatin was completely dissolved, then further cooling in water bath at 10°C for 16-18h. Gel strength of resulted sample was determined by Bloom gelometer (Stevens, LFRA, USA) expressed as bloom (grams).

Bloom strength may be defined as the weight in grams necessary to apply to the surface of a gel by means of a piston 12.7mm in diameter, in order to produce a 4mm deep depression. Commercial products normally have gel strength between 50 and 300 bloom (grams). Gelatin with strong gel strength named frot bloom but with weak gel strength named faible bloom.

Viscosity Determination: For viscosity determination gelatin samples was prepared in water bath at 45°C until completely dissolved then poured to the beaker for viscosity was determined by rotary viscometer (HAAKE,VT-02,USA). Viscosity expressed as centipoise or pa.s [19].

Statistical Method: This experiment was carried out a randomized completely design with 3 replication. Treatments were fitofague's acidic gelatin and Fitofague's alkaline gelatin All recorded data was analyzed with SAS(ver.9.1) software.

RESULTS AND DISCUSSION

The comparison of yield, physiochemical and rheological properties of acid and alkaline Fitofague's gelatin were presented in table 1. Results shown that the yield of acidic gelatin (7.5%) higher than alkaline gelatin (6.5%) but quality factors of alkaline gelatin was better than acidic gelatin.

Gelatin was extracted from fitofague's skin and fin with acidic and alkaline methods were compared with national standards (ISIRI) that presented in table 2 and international standards presented in table 3 [15, 20]. Results shown that characteristics of extracted gelatin sought to national and GMIA standards. In comparison of quality factors in table 3 shown that fitofague's acidic gelatin can be used in food application and tablet production but due to it's lower gel strength (84 bloom) relative to soft (150-200) and hard capsules (240-300) doesn't recommended in their production.

Table 1: Comparison of yield, physiochemical and rheological properties of fitofague gelatin

Quality factors	Fitofague acidic gelatin	Fitofague alkaline gelatin
Moisture content(%)	10.10	9.50
Ash(%)	2.2	2.07
Nitrogen(%)	15.76	16.00
Protein(%)	86.03	86.17
pH	5.2	7.1
Setting temperature(C)	7	10
Setting time(s)	180	135
Melting temperature(C)	20	26
Melting time(s)	60	150
Viscosity(cp)	4	6
Bloom gel strength(g)	84	176
Color	---	---

Also with comparison of Fitofague's alkaline gelatin with GMIA standards in table 3 concluded that it is useful in food application but due to it's higher gel strength(176) relative to tablet (75-150) and soft capsules (125-175) and lower gel strength relative to hard capsules (200-250) doesn't recommended in pharmaceutical industries.

Comparison of acidic and alkaline fitofague's gelatin quality factors with acidic and alkaline mammalian gelatin shown at table 4 [10]. Results shown that their quality factors near to pig and calf gelatin but their setting and melting temperature are different. The main reason for this difference due to amino acids profile of fish gelatin. Table 4 shows a comparison of the amino acid composition of carp fish gelatin with other sources. Glycine represents a third of the total amino acids residues. There are similarities in the composition, but what is important, is the total amount of proline and hydroxyproline [21].

Although all gelatins are composed of the same 20 amino acids but it is important to note that the uniqueness of fish gelatin lies in its amino acid content. Fish gelatin has lower amounts of imino acids (proline and hyroxyproline) which are important during the hydrogen bonding of gelatin in water solutions. This results in a reduction of the gelling temperature. An example in this case is that alkaline gelatin from Fitofague skins and fins gels at 10°C, while gelatin was extracted from animal based sources gels above room temperature (Table 5). As mentioned earlier, fitofague's gelatin quality was extracted with alkaline method is higher than acidic method, while both have higher quality in comparison of other sources.

In conclusion, gelatin could be extracted successfully from skin and fin of silver carp fish (Fitofague) with acidic and alkaline methods. Yield of acidic gelatin is more than alkaline gelatin but quality factors of alkaline gelatin are higher than acidic gelatin. Their quality factors sought to national and international standards because gel strength of acidic gelatin is lower than alkaline gelatin, it has cheaper price and could used to some applications such as fruit juice clarification.

Table 2: Comparison of some physiochemical properties of fitofague gelatin with national standards

Gelatin Type	Moisture content ((%)	Ash (%)	Nitrogen (%)	Color
Acidic gelatin	10.10	2.2	15.76	---
Alkaline gelatin	9.50	2.7	16.00	---
Edible gelatin standards	Max.15	Max.3	Min.15	Pale yellow to amber

Table 3: Comparison of quality factors of fitofague's gelatin with GMIA standards

Gelatin Type	Moisture Content (%)	Ash (%)	Protein (%)	Viscosity (cp)	Bloom gel strength (g)	pH
Fitofague acidic gelatin	10.10	2.20	86.03	4	84	5.2
Fitofague alkaline gelatin	9.50	2.07	88.17	6	176	7.1
Food grade acidic gelatin standard	8-15	1-2.5	84-90	1.5-7.5	50-300	3.8-5.5
Hard capsules acidic gelatin standard	8-15	1-2.5	84-90	4.4-5.5	240-300	4.5-5.5
Soft capsules acidic gelatin standard	8-15	1-2.5	84-90	2.5-3.5	150-200	4.5-5.5
Tablet acidic gelatin standard	8-15	1-2.5	84-90	1.7-3.5	75-150	4.5-5.5
Food grade alkaline gelatin standard	8-15	1-2.5	84-90	2-7.5	50-300	5-7.5
Hard capsules alkaline gelatin standard	8-15	1-2.5	84-90	4.5-6	200-250	5.3-6.5
Soft capsules alkaline gelatin standard	8-15	1-2.5	84-90	3-4.5	125-175	5.3-6.5
Tablet alkaline gelatin standard	8-15	1-2.5	84-90	2-3.5	75-150	5.3-6.5

Table 4: Amino acid compositions of fish, porcine and calf gelatins (Residues/1000 Amino Acids)

Amino Acid	Silver carp waste gelatin	Porcine skin gelatin	Calf skin gelatin
Alanine	120	114	112
Glycine	317	328	320
Valine	19	18	20
Leucine	25	20	25
Isoleucine	12	9.2	11
Proline	124	129	138
Hydroxyproline	73	70	94
Phenylalanine	14	14	13
Tyrosine	3.2	1.8	2.6
Serine	43	41	36
Threonine	27	25	18
Methionine	12	12	4.3
Lysine	27	22	27
Hydroxylysine	4.5	7.9	7.4
Cystine	Trace	Trace	Trace
Histidine	4.5	7.4	5
Arginine	53	45	50
Aspartic Acid	47	54	45
Glutamic Acid	74	81	72

Table 5: Comparison of quality factors of Fitofague's gelatin with mammalian gelatin

Quality factors	Fitofague's acidic gelatin	Mammalian acidic gelatin	Fitofague's alkaline gelatin	Mammalian alkaline gelatin
Bloom gel strength (g)	84	75-300	176	75-275
Viscosity (cp)	4	2-7.5	6	2-7.5
Setting temperature (°C)	7	15-29	10	15-29
Melting temperature (°C)	20	27-32	26	27-32
Ash (%)	2.2	0.3-2	2.07	0.05-2
pH	5.2	3.8-6	7.1	5-7.4

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