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Extraction and evaluation of gelatin from yellow fin tuna (*Thunnus albacares*) skin and prospect as an alternative to mammalian gelatin

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Abstract

One of the mainly popular consumed colloid protein materials in pharmaceutics, medical, food and military industries is Gelatin. Especially from warm-water fish gelatin report poss similar characteristics to mammalian's gelatin .Yellow fin tuna (*Thunnus albacares*) gelatin skin, lots of waste in form of skin and bones of the fish are produced every day. Analysis factors were extracted alkaline gelatin from skin, physiochemical and rheological test (amino acids composition, SDS- page electrophoreses, FTIR (Fourier transform infrared), moisture content, pH, setting point and setting time, melting point and melting time, color and gelatin yield). In contrast cool water fish gelatin, yellowfin tuna had higher gelatin content (Proline and Hydroxyproline) than mammalian gelatin content. SDS-electrophoresis for yellow fin gelatin showed protein bands (α, β, γ) same as mammalian's protein bands. FTIR (Fourier transform infrared) had the same spectra for both of them. Factors were pH (6.1), Moisture (8.5%) Setting temperature and time respectively 4(c) and 60 (s) and Melting temperature and time respectively were 50 (c) and 45 (s). The color was transparent. In light of these results yellow fin tuna prospect as an alternative to mammalian's gelatin.

Keywords: Rheological, Yellow fin tuna, Fourier transform infrared, Proline and Hydroxyproline.

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Introduction

In food, pharmaceutical, cosmetic, and photographic applications, one of the most popular biopolymers, is Gelatin. Gelatin is widely used, due to functional and technological properties. (Riaz and Chaudry, 2004; Gimenez *et al*., 2005; Karim and Bhat, 2009).In medical and pharmaceutical fields, gelatin is used as a matrix for implants in intravenous infusions, and in injectable drug delivery micro spheres (Saddler and Horsey, 1987; Pollack, 1990; Rao, 1995).In the food industry, gelatin is utilized in confectionery, low-fat spreads, dairy, baked goods and meat products (Johnston-Banks, 1990; Schrieber and Gareis, 2007). There are some reports showed that in which live attenuated viral vaccines used for immunization against measles, rubella mumps, rabies, diphtheria, Japanese encephalitis, and tetanus toxin contain gelatin as a stabilizer (Burke *et al*., 1999). In the pharmaceutical industry, gelatin is generally used for the manufacture of soft and hard capsules, plasma expanders, and in wound care. Gelatin, being low in calories, is normally suggested for utilize in foodstuffs to enhance protein levels, and is especially useful in body-building foods. In addition, gelatin is also used to decrease carbohydrate levels in foods formulated for diabetic patients. Inured, there are two process to acquire gelatin, an acid process (gelatin A with isoelectric point at pH 5) and an alkaline process (gelatin B with isoelectric point at pH 6-9) Gelatin from marine sources (warm and cold water fish skins, bones and fins) is possible alternative to mammalian's gelatin (Rustad, 2003; Kim and Mendis, 2006; Wasswa *et al*., 2007). From yellowfin tuna skin extracted gelatin. The yield and quality of gelatin are not only influenced by the species or tissues from which it has been extracted, but also by the extraction process itself (Montero and Gomez-Guillen, 2000). Gelatin is derived from Collagen which is a major structure protein found in skin and bones of animals. Commercial gelatin is mostly extracted from pigs and cows (mammalian's) skins and bones. However, the use of gelatin from those resources is limited albeit the outbreaks of bovine spongiform encephalopathy (BSE) or mad cow disease and religious reasons. This conditions because of religious emotion (both Judaism and Islam forbid the consumption of any pork-related products and Hindus do not consume cow-related yield) In addition to the improved and stricter observance to vegetarianism all over the world (Wilesmith *et al*., 1991). Hence, there is an increasing concerns in the production of fish gelatin as an alternative for mammalian matching part (Jamilah and Harvinder, 2002). For food applications, the most important properties characterizing gelatin is melting points. This property is affected by many factors, such as the average molecular weight and molecular weight distribution. (Norland, 1990; Osborne *et al*., 1990; Choi and Regenstein, 2000). Properties physicochemical and rheological warm water fish' s gelatin is similar to mammalian's gelatin. These gelatin extracted from skin (waste

form) was cheaper than mammalian's gelatin. Yellowfin tuna (*Thunnus albacares*) is a good indicator of warm-water fish. Due to the amount of catch of yellowfin tuna in the Oman Sea, lots of waste in form of skin and bones of the fish are produced every day. Conversion of such a waste in to a value-added product would help strengthen the economy in the industry. Here, gelatin has been extracted from yellowfin tuna skin, and then compare with mammalian gelatin.

Materials and methods

The yellowfin tuna resides in Oman Sea. The dorsal skin of yellowfin tuna was the material under study and used in this research.

Extraction of type B gelatin (alkaline) from skin

The yellowfin tuna skin was washed, chopped and kept frozen at -18 $^{\cdot}C^{o}$ until use. The cleaned skins were treated 8 volumes (v/w) of alkaline solution (1-3% NaOH) at 10 C^{o} in shaking incubator at 200 rpm (554-D, Sahand azar Azar Co, Iran) for 2 hours in order to remove the subcutaneous tissues and non-collagen proteins after they were swollen. After the alkaline treatment, with 6N HCl neutralized the skin and washed in hot water preparing for extraction. Six volumes (v/w) of distilled water were added and heated in temperature ranging 60 ^{o}C for 1 to 9 hours. The extracted solution was centrifuged for 25 minutes at 2500rpm at 25 C^{o}. The upper phase would then be put in to sterile plates and heated at 60 C^{o} for 24 hours (Shimaz Co, Iran)(Cho *et al*., 2005).

Some physiochemical and rheological tests

pH measurement

pH was determined by pH meter (Sana pH. MV.TEM/METER 01-091) according to Iranian Standard 3474.

Color determination

Color of gelatin samples were measured by putting them on white background and compared with each other. According to Iranian Standard 4374.

Moisture content

Moisture content was determined, according to Iranian standard 3474.

Setting point and setting time

Setting point and setting time were determined according to Muyonga *et al*., 2004. For setting point determination, first 10 %(w/v) gelatin solution was made in warm water bath and then 30mL transfer to the test tube (12mm$*$ 75mm) later set the water bath at 40 ^{o}C. Then cooling water bath slowly added of cold water at 2 ^{o}C for time breaks 15 s. Thermometer put in the solution and out each 15s until no drop did not drop anymore; this temperature was recorded as gelatin setting point.

Melting point and melting time

Melting point and Melting time were determined according Muyonga *et al*., 2004. 10 %(w/v) gelatin solution was set like past section, placed to refrigerator at 3 ^{o}C for 6 hours then

relocation to water bath at 10 ^{o}C with slowly addition of warm (45 ^{o}C) and melting temperature and time were recorded.

Gelatin yield

The ratio of dried gelatin weight to the total fish bones weight on wet basis was used as the gelatin yield.

Yield of gelatin (%) = (weight of dried gelatin [g] / wet weight of fresh skin [g]) × 100

Electrophoresis analysis

The gelatin of yellowfin tuna was determined by sodium dodecyl sulfate polyacryamide gel electrophoresis (SDS-PAGE) according to method of Laemmli (1970). The sample (1 g) were mixed in 10 mL of 5% (w/v) SDS solution. The combination has been warmed at 85 ^{o}C for 1h in a water bath to became a solution total proteins. Supernatants were gathered after centrifuging at 3000g for 3min. Then blended with sample buffer (0.5M Tris – HCl, pH 6.5 containing 4%(w/v) SDS and 20% (v/v) glycerol at the ratio of 1:1(v/v).Samples (20 µg protein) were loaded in to polyacrylamide gel made of 5% running gel and 4% stacking gel and subjected to electrophoresis at constant current of 15mA per gel using a Mini protein ÍÍ unit. Bovine skin gelatin (Merck) was used as a standard gelatin. Used ladder for determination molecular weight. After electrophoresis, staining the gel did with 0.5% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid.

Fourier transforms infrared (FTIR) spectroscopy

Spectra of gelatin from yellowfin tuna skin were using simple FTIR (8400s-CHIMA DZU IRAN) by Pavia method (Pavia, 1993)1

Analysis of amino acids

Amino acids composition of the samples is determined by using of the high performance liquid chromatography (HPLC) method (Games, 1987).

Result

Gelatin was extracted from skin with alkaline method were compared with national standards (Iran national standard,1994)

Some quality factors

Gelatin is extracted from yellow fin's skin with alkaline method. Table 1 shows some physiochemical and rheological properties of yellowfin tuna gelatin then compared with national standard (ISIRL) that presented in tuna gelatin and international standards presented in (Table 3). In (Table 2) The Results showed that comparison of quality factors of yellowfin tuna with GMIA standards.

1.Which was made of bromide potassium had 45.^{o} angle of incidence to the IR beam. The resolution of 4cm^{-1} was acquired at room temperature and the measurement range was 4000-600cm^{-1}(mid IR)

Table 1: Some physiochemical and rheological properties of yellowfin tuna gelatin.

Quality factors	Yellowfin tuna gelatin
Moisture content (%)	8.5%
pH	6.1
Setting temperature(c)	4
Setting time(s)	60
Melting temperature(c)	50
Melting time(s)	45
Color	Transparent

Table 2: Comparison of quality factors of yellowfin tuna with GMIA standards.

Gelatin type	pH	Moisture content (%)
yellow fin tuna gelatin	6.1	8.5%
Food grade alkaline gelatin standard	5-7.5	(8-15)%
Hard capsules alkaline gelatin standard	5.3-6.5	(8-15)%
Soft capsules alkaline gelatin standard	5.3-6.5	(8-15)%

Table 3: Comparison of some quality factors of Yellowfin tuna's gelatin with mammalian (national standard).

Quality factors	Yellowfin tuna gelatin	Mammalian alkaline
Color	Transparent	Pale yellow to amber
pH	6.1	5-7.4
Setting temperature(c)	4	15-29
Melting temperature(c)	45	27-32
Moisture (%)	8.5	(8-15)%

Gelatin yield

The extracted gelatin from yellow fin tuna skin in its from amount of 10 g descaled skin, extracted gelatin was0.99 g obtained.

SDS-PAGE analysis

The protein patterns of gels from yellowfin tuna and bovine skin gelatin were shown in (Fig. 1)

Evidently, the electrophoresis pattern of extracted gelatin was essential similar to that, the commercial gelatin from bovine skin. In addition, α1 and α2 chains were found as the major components for both types of gelatin (molecular weight of about 130KD). A considerable presence of β-component (α-chain dimmer), and particularly of higher molecular weight polymers including γ-components (α-chain trimmers)(molecular weight of about 250KD) β-component and γ-component in yellow fin tuna gelatin is similar to commercial gelatin from bovine skin.

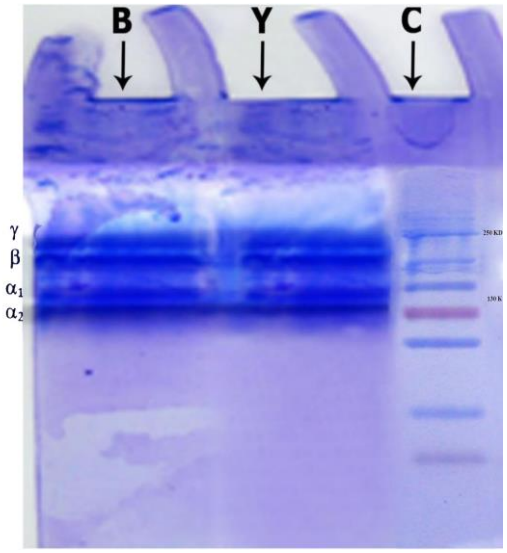

Figure 1: SDS-PAGE pattern gel from Yellowfin tuna (Y) and bovine skin (B) ladder for molecular weight) (250&130KD) (C).

FTIR spectra of gelatin

FTIR spectra of gelatin extracted from skin shown in Fig. 2. FTIR spectroscopy has been used to study changes in the secondary structure of gelatin. Spectra dorsal skin gelatin displayed major bands at 3303.83 cm^{-1} (Amide A representative of NH-stretching, coupled with hydrogen bonding), 1652.86 cm^{-1} (amide I, representative of C=O stretching /hydrogen bonding coupled with COO-) 1539.09 cm^{-1} (Amide II, representative of NH bending, coupled with CN stretching) and 1252 cm^{-1} (amide III representative of NH bending). FTIR spectra of yellowfin skin gelatin were similar to those found in other gelatins (Muyonga *et al*., 2004)

Wave numbers (cm^{-1})

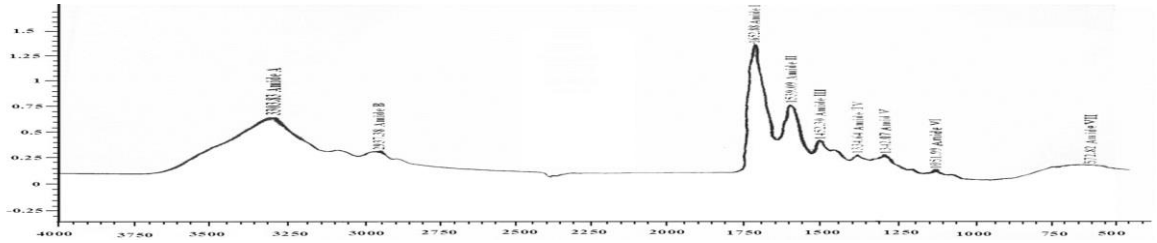

Figure 2: Fourier Trans form infrared (FTIR) spectroscopic spectra of gelatin extracted from yellowfin skin.

Amino acids composition

The amino acids composition from yellowfin tuna skin`s gelatin was shown in Table 4. Glycine was major component (22.4 g $100g^{-1}$ protein for gelatin from yellowfin tuna skin. Relatively high contents of Proline, Hydroxyproline were 19.6 and 6.35 g $100g^{-1}$, respectively. Glutamic acid and arginine and other amino acids for yellowfin tuna skin gelatin were observed.

Table 4: The amino acids composition from yellow fin tuna.

Amino acid	Yellowfin tuna skin gelatin
Alanine	9.08
Arginine	7.06
Aspartic acid	4.82
Glutamic acid	8.81
Glycine	22.44
Histidine	0.65
Hydroxyproline	6.35
Isoleucine	1.18
Leucine	2.30
Lysine	3.77
Methionine	2.09
Phenylalanine	2.14
Proline	19.60
Serine	2.38
Threonine	3.38
Tyrosine	0.47
Valine	1.9

Discussion

Comparison of alkaline yellowfin's gelatin quality factors alkaline mammalian gelatin shown at Table 3 and properties alkaline yellowfin tuna gelatin shown at Table 1. Results shown that their quality factors near to mammalian gelatin but their setting and melting temperature are different. The main reason for this difference due to amino acids profile of fish gelatin Different yield values for the gelatins extracted from other fish skins were reported in the open literature: some of these were for black tilapia (5.4%), red tilapia (7.8%) (Jamilah and Harvinder 2002), young Nile perch (12.3%), adult Nile perch (16.0%)(Muyonga et al., 2004)sin croaker (14.3%), shortfin scad (7.3%),bigeye snapper(6.5%), and brown stripe red snapper (9.4%) skins (Jongiareonrak *et al*., 2006). The observed smaller yield for the gelatin extracted from Nile tilapia skin than those for the gelatins extracted from young Nile perch, adult Nile perch and sin croaker skins and greater than black tilapia, red tilapia, Shortfin scad, bigeye snapper and brown stripe red snapper were noted and the difference in such values depends on the differences in the proximate composition of the skins, the collagen content (Jongiareonrak *et al*., 2006), and the amount of soluble components in the skins (Muyonga *et al*., 2004), as these properties vary with the species and the age of the fish, as well as the difference in the extraction method.

About SDS-PAGE analysis, fish and mammalian gelatins have a polydesperse molecular weight distribution related to the collagen structure and production process. Gelatin from yellowfin tuna was similar to commercial gelatin (B) bovine skin. Both type gelatins had α, β, γ components with similar molecular weight. The extraction process can affect the efficient properties and the length of the polypeptide chains in gelatin. This depends on the processing parameters (temperature and time), the pretreatment, properties and protection method of the starting raw material. Alkaline treatment is suitable for the more complex collagens made with bovine hides. Furthermore different oligomers of the alpha subunits, intact and partially hydrolyzed alpha-chains were also present, making rise to a mixture containing molecules of different molecular weights (Schrieber and Gareis, 2007). Large amounts of β - and γ -chains have been shown to negatively influence on some of the functional properties of fish gelatins, such as, lowering melting, lowering viscosity and setting points, and resulting in a longer setting time (Muyonga *et al*., 2004; Cho *et al*., 2006). Gelatin extracted from both *P. tayenus* and *P. macracanthus* contained α1 and α2-chains as the major components were characterized to be same type (Benjakul *et al*., 2009). The intensity of β-chain (α-chain dimmer) were α1 and α2 chains and it had the proteins peptides with molecular weight lower than the temperature and time. Complete degradation of gelatin was observed at 120 (min) and 10 ˚C of extraction in the change of collagen to gelatin might provide the molecules

with varying chain length, mainly due to cleavage of inter-chain covalent cross-link unfavorable breakage of some intra-chain peptide linkage (Muyonga *et al*., 2004; Zhao *et al*., 2007). In present study γ – andβ- chains exactly were similar to mammalian`s gelatin and it help functional properties of yellowfin tuna`s gelatin. Complete degradation of yellowfin tuna`s gelatin was observed at 9h and 60 ˚C of extraction. Molecular weight distribution of Hake and Flat fish are generally characterized by presence of β-components and higher molecular weight forms, as well as α1 and α2 chains (similar as tuna fish) (Gimenez, *et al*., 2005; Carvalho *et al*., 2008) fish skin gelatins reported that pollock and salmon gelatins had slightly different molecular weight profiles compared to porcine gelatin, and that the fish gelatins had chains with slightly lower molecular.

About FTIR spectra in study secondary structure of proteins and polypeptides, Nine characteristics of FTIR absorption band, namely A, B and I, VII could be observed in a typical IR spectrum of which amide I band (1700-1600cm^{-1}) was most sensitive and widely usage in studies of protein secondary structure. Amide I band was mainly because of C=0 stretching vibration (about 80%) of the amide group coupled with in plane NH bending (less than 20%) (Kong and Yu, 2007). Amide II (1575-1480 cm^{-1}) obtains mostly from in plane N-H bending and C-N stretching vibration and are less protein conformational sensitivity compared to amide I, while other amide vibration bands had less practical use in protein conformational studies (Kong and Yu, 2007). Amide I and II bands of gelatin from Yellow fin tuna`s skin were at the wave number of 1652.86 and 1539.09 cm^{-1}(Fig. 2). Higher frequencies of amide I bands was attributed to greater loss of molecular order of triple helix due to uncoupling of intermolecular cross-links and disorganization of intra molecular bonding when gelatin was extracted long time at higher temperature (Kittiphattanabawon *et al*., 2010; Ahmad and Benjakul, 2011). In addition, amide III band of yellow fin tuna`s gelatin was detected at 1242 cm^{-1} which was related loss of transformation of α-helical to random coil structure due to denaturation of collagen to gelatin and triple-helix state of molecules (Muyonga *et al.*, 2004). Amide III band of gelatin at 1237cm^{-1} was reported in big eye snapper (Benjakul *et al*., 2009). Amide A band derived from the stretching vibration of N-H group (Kong and Yu, 2007).In this study, N-H stretching band appeared at 3303.83cm^{-1}. N-H stretching vibration of amide A occurred normally at wave number of 3440-3400cm^{-1} (Muyonga *et al*., 2004). When N-H group of shorter peptides were included hydrogen bonding, the band in amide a region shifts to lower frequencies. Amide A band of yellowfin tuna skin`s gelatin treated with hydrochloric acid for 2 hours. Indicating the included of N-H group of shorter peptide fragments in hydrogen banding (Ahmad and Benjakul, 2011). Amide B band derived from the stretching N-H and bending C-

H. Amide B changes with temperature parameters and acid concentration. If it had high acid concentration, it would be wide peak. It prejudiced by hydrogen band.

Furthermore about the amino acids compositions of gelatin from yellowfin tuna and porcine skins are summarized in Table-5. For both type of gelatins, glycine was major component (22.4 g 100 g^{-1} protein for gelatin from yellowfin tuna`s skin and 22.4 g $100g^{-1}$ protein for gelatin from porcine skin), followed by alanine (i.e., 9.08 g $10g^{-1}$ protein for gelatin from yellowfin tuna skin and 12.55 g $100g^{-1}$ protein for gelatin from porcine skin). Relatively high contents of Proline, Hydroxyproline, glutamic acid and arginine and amino acids for both types of gelatin were observed .The highest content of glycine in gelatin was logical, as glycine was required at every third position of protein because the assembly of the triple helix of collagen puts this smallest amino acids residue at the interior of the helix (Anonymous, 2007). Evidently, the amino acid content in the gelatin from yellowfin tuna skin (i.e., 25.95 g $100g^{-1}$ protein) was higher than that in the gelatin from porcine skin (i.e., 19.3 g $100g^{-1}$ protein) and also they reported that the amino acids contents on gelatin from sin croaker, shortfin scad skin (i.e.,11.8 and 10.0 g $100g^{-1}$ protein) and gelatin from commercial bovine (i.e., 13.7 g $100g^{-1}$ protein). The amino acids content (Proline and Hydroxyproline) in the gelatin from yellowfin tuna skin was higher than others and these two amino acids thermally stabilize the triple helix of collagen also ordered conformation when gelatin forms a gel network. The lower content of Proline and Hydroxyproline gave fish gelatin a low gel modulus, and low gelling and melting temperatures. It should be kept in mind that the super-helix structure of the gelatin gel, which was critical for the gel properties, was stabilized by steric restrictions. These limits were forced to both the pyrrolidine rings of the amino acids furthermore to the hydrogen bonds formed between amino acids residues (Te Nijenhuis, 1997). Together with Proline and Hydroxyproline were found in non-polar regions where sequences of the type Gly-Pro-Y predominate, with the third position normally engaged by Hyp (Ledward, 1986). Therefore, in generally, a gelatin preparation with high Pro, Hyp, Gly content showed better viscoelastic properties than others with a lower content of these amino acids, according to (Table 5).

Table 5: Amino acids composition of extracted gelatin from Nile tilapia skin, commercial gelatin from porcine skin and gelatin from yellow fin tuna.

Amino acid	Content (g $100g^{-1}$ protein) Nile tilapia skin`s gelatin	Porcine skin`s gelatin	Yellowfin tuna skin`s gelatin
Alanine	11. 89	12.55	9.08
Arginine	8.71	7.43	7.06
Aspartic acid	8.20	7.84	4.82
Glutamic acid	8.99	8.46	8.81
Glycine	21.18	22.45	22.44

Table 5 continued:

Histidine	0.20	0.20	0.65
Hydroxyproline	8.70	9.46	6.35
Isoleucine	0.88	1.06	1.18
Leucine	2.12	2.32	2.30
Lysine	3.02	3.42	3.77
Methionine	1.13	0.82	2.09
Phenylalanine	1.74	1.66	2.14
Proline	8.83	9.80	19.60
Serine	3.96	3.18	2.38
Threonine	5.82	5.92	3.38
Tyrosine	0.67	0.81	0.47
Valine	1.67	1.98	1.9

In light of these results, we conclude that the use yellowfin tuna`s gelatin as alternative to mammalian`s gelatin. Yellow fin tuna had higher gelatin content (Proline and Hydroxyproline) than mammalian`s gelatin content. SDS-electrophoresis for yellowfin gelatin showed protein band (α,β,γ) same as mammalian protein band. FTIR (Fourier transform infrared) had the same spectra for both of them. pH was 6.1.Moisture was 8.5%. Setting temperature and time respectively were 4(c) and 60(s). Melting temperature and time respectively were 50(c) and 45(s). The color was transparent. These data is same as mammalian`s gelatin.

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