International Research Journal of Applied and Basic Sciences
© 2014 Available online at www.irjabs.com
ISSN 2251-838X / Vol, 8 (7): 904-910
Science Explorer Publications

# Comparison of collagen extracted from the skin and fin of long tail tuna Thunnus Tonggol

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**ABSTRACT:** This study was conducted i to extract collagen protein from the skin and fin of longtailtuna Thunnustonggol. During fish processing, a great deal of wastes including water and solids were produced and the skin and fin of fish, arising from wastes of aquatic processing, can be considered a replacement of gelatin and collagen sources. Samples used in this study were collected from fish conservation company of Bandarabbas, Iran. In an experiment for collagen extraction from every area of skin and fin was studied and analyzed chemically 3 times. Additionally, the amount of humidity, ash, fat, protein and amino acids were evaluated in these sections. The results showed that in the skin of the tuna has the most percent of humidity, while it didn't have a significant difference with the humidity of fin ($P>0.05$). Skin has the most amount of protein ($P<0.05$). In both samples of skin and fin of the tuna, glycine amino acid was the dominant amino acid and hydroxyprolineamino acid (the main indicator of collagen) in skin showed higher amount in respect to fin ($P<0.05$).According to achieved results, it can be said that the skin of the tuna fish has more amount of collagen compared to fin ($P<0.05$). Moreover, electrophoresis model indicated that there isn't any difference in collagen of skin and fin of the tuna ($P>0.05$) and both obtained collagen have at least two chains of α 1 and α 2.
***Keywords:*** collagen, tuna,Thunnustonggol, skin, fin.

## INTRODUCTION

During fish processing, a great deal of wastes including water and solids are produced (Morrissey et al., 2000). Solid wastes include 50-70% of raw materials and they consist of a synthesis of different parts, head, intestine, viscera, and bone depending on used process (Shahidi, 1994; Bailey et al., 1989; Morrissey et al., 2000). In general, the skin and bone of cow and pig are major sources of gelatin and collagen extraction. The outbreakof mad cow disease contributes to anxietyamongconsumers of cow gelatin (Shahidi, 1994). Consequently, the skin and fin of fish arising from wastes of aquatic processing can be considered as gelatin and collagen sources. About 30% of aquatic wastes processing include skin which is rich of collagen (Shahidi, 1994; Bailey et al., 1989; Morrissey et al., 2000). The nutritional value of skin and fin of fish is high and these materials are helpful sources for producing fish meal, but according to reduction of fish stocks and excessive hunting around the world, there is a possibility for wastes' reduction (Nagai et al., 2000).nowadays, various researchers have studied collagen removal of fish wastes. (Kimura, Miyauchi& Uchida, 2004; Kimura, Omura, Ishida and Shirai, 2001; Nomura, Sakai, Ishii &Shirai, 2000; Omura, Urano and Kimura, 2002,Nagai et al., 2002) stated that in collagen extraction of outer skin of sepia lycidasspecies, primary extraction had 2% of dry weight of collagen and indicates a simple alpha bandand it seemed that α was 1. In addition, a large number of beta chains have been achieved in PSC. Collagens are weak in intersection components of inter and intra molecular.

Studies have indicated that maximum properties of collagen type 1 gelling and viscosity are achieved when fish skin is diluted besides sodium-hydroxide (Gomez guillen, 2000). And the capacity of proline amino acids and hydroxyproline of collagen is closed to thermal stability in black and sheep head fish. In addition, the collagen of half tropical fishes has more thermal stability (Ogawa, 2003). Studies indicate that the skin of grass carp fish which includes glycine 1.3 is considered as main amino acid and very low amount of methionine, tyrosine and histamine like other collagens and proline amino acid and hydroxy proline of skin collagen were 186 proline amino acid and

hydroxyprolineof 1000 amino acids (Ikoma et al., 2005). The collagen is used in medicine for producing creams, gas for dressing and wound healing and in pharmacy is used for producing medicinal capsules and tablets. Furthermore, collagen plays a crucial role in photography and creates an emolusion of silver salt which is very sensitive to light (Shahidi, 1994; Bailey et el., 1989; Morrissey et al., 2000; Ishida &Shirai, 2001; Nomura et al., 2004; Kimura et al., 2002). Fish collagen has economical value as the source of fish glue, but it is not provided in large scales. Due to high level of solubility, the collagen of fish swim bladder is the first found collagen solution. Moreover, the collagen of caviar swim bladder is still commercially used in refining of alcoholic drink. The collagen of fish skin solution is studied extensively and unusually three its major chains are all different (Shahidi, 1994; Bailey et el., 1989; Morrissey et al., 2000). In fact, fish's collagen has less amino acid comparing to mammal's collagen. Also, the collagen of shark skin solution and cartilaginous fish have been achieved which is similar to spine scent's collagen (Nagai et al., 2000). Perhaps alastodine is the most interesting collagen discovered in aquatics which is produced from protein of shark's fin. This protein has less molecular weight comparing to most collagens of other solutions and its high amount of tyrosine and cysteine is abnormal. These two amino acids play role in connection formation, but this phenomenon has not been seen in mammal's collagen (Morrissey et al., 2000). In Iran, annual fishing of tuna and pseudo-tuna (such as Narrow-barred Spanish mackerel and indo-Pacific king mackerel) is about 178000 tons in which tuna allocates 50% in Hormozgan, Iran (Iran Fishery Organization, 2010: longtailtuna fish is one of the most important industrial fishes of Persian Gulf and Oman sea which is economically crucial for Iran fishery industries).

So far many studies are performed on collagen among which it can be addressed to biochemical properties of extracted collagen of skin and bone of onchorhynchusmykiss that acidic collagen (ASC) has been removed of salmon skin and bone for optimal usage of fish byproduct by HodaShahiriTabarestani in 2008. The yield of collagen extraction about skin and bone were 9.448% and 1.122% according to wet weight. On the basis of electrophoresis model, both types of collagen as collagen type I were categorized according to the amount of relatively low amino acid and different amino acid compounds. The collagen of skin and bone had the least solubility in pH, which is 9 and 7, respectively. The salt density doesn't make a change in collagen solubility to 3%, but there was seen a severe reduction higher amount salt more than 3%.

In addition, collagen extraction of cod fish was under the influence of reaction and chemical performance based on using method of 0.7% citric acid and low density of sulfuric acid and sodium hydroxide which had the most efficiency and Gudmundsson et al., 1997 reported the resulting collagen as 17.26%.

In 1998 in America, some evaluations were conducted on collagen type I extraction by Ohno& Kimura and the percent of acidic collagen type I was indicated at 0.0844%. However, no report based on collagen extraction of dried waste of tuna fish is reported. In this study, it is preceded to collagen protein extraction, fin and skin of longtail tuna. Additionally, in this section the amount of humidity, ash, fat, protein and amino acid are compared.

## MATERIALS AND METHODS

In order to collagen separation, the dried wastes samples were selected randomly according to qualitative properties of fresh fish and a certain volume of wastes were provided from Bandarabbas fishery products organization. The experiment's stages were conducted in ecology laboratory of Persian Gulf and Oman Sea research center.

In this experiment, wastes were divided into two treatments including skin and fin and each treatment was evaluated three times by considered indicators. For testing, all materials were kept in fridge at 18 $^{o}$C for one week. Then the samples were moved to factory's laboratory and they were there in the same status till the experiment begins. During the process, temperature condition was 4 $^{o}$C and pH 7.2 was constant and controlled (Negai et al., 2000).

For experiment, 50 grams of each sample was weighted and afterbeing exited of freezing status, they were weighted again in room temperature and their lost water was calculated. Then skin and fin of fish were taken into small pieces and stirred in normal 1.0 caustic soda for 6 hours with magnetic stirrer to remove stirred non-collagen proteins to be extracted. Then it was washed with distilled water. In order to separate collagen from skin, the first stage, fat removal of skin, was performed in which the skin was put in 10% butyl alcohol, after being passed the mentioned time, the samples were washed by distilled water. Then, insoluble material was placed in 0.5 molar acetic acid for 3 days and the obtained extract was extracted by centrifuging for 1 hour with 20000 rpm.The remained material was placed again in 0.5 molar acetic acid for 2 days in order to be extracted again, the salt is added gradually to the final solution for reaching of final density to 0.9 molar. In this process, the collagen is gradually resides and extracted (Nagai et al., 2000).

In order to remove collagen from fins, their insoluble materials are placed in0.5molar aceticacid for 3 days, after being passed the mentioned time, it will centrifuge with 20000 rpm for 1 hour. The obtained materials will be divided into two parts, one insoluble in acid and the other soluble in acid; the collagen is in insoluble part (Nagai et al., 2000). In this step, the above mentioned part is washed in distilled water and is placed in 0.5 molar EDTA for 5 days in order to remove calcium, hereafter the remained materials are washed in distilled water. In order to remove the fat of obtained sample, place it in 10% butyl alcohol for 1 day, after the mentioned time, the sample was washed in distilled water. Finally, the remained materials were extracted by centrifuging of 20000 rpm for 1 hour. The remained of substance sediments were solved in 0.5 molar acetic acid to become a solvent and at the end salt was added by which collagen separated. Other stages of sample analysis were conducted after providing of polyacrylamide-SDS (Samboork, 1989). The collagen sample was solved in 0.02molar sodium phosphate (pH= 7.2), 1% SDS and 3.5molar urea. Electrophoresis on 5.3 gels was performed in buffer of 0.1molar phosphate (pH= 7.2) containing 1% SDS. After gel production, two-dimensional photo considered in electrophoresis was analyzed.
After gel preparing for its painting, place the gel in a solution ofdistilled water containing ethidium bromidefor 10 minand thenwashedwith purifieddistilled water and then observe the bandsof collagen protein bygel dactiometerdevice by UV light.

In desired method of solution making for two-dimensional electrophoresis, all non-covalent bonds must be broken in the protein complexes and protein aggregations transform to single and solution poly peptides. Samples' collapse must be done in chill and during the process, considered sample should be kept over ice. For protectingthe protein sample from released proteinase action during collapse of cells, some arrangements should be considered. One of the common methods of direct cell's collapse in lubricating solutions is that quickly stop the proteinases and other enzyme actions, these solutions have some powerful denatured materials.

In this study, copper sulfate, sodium sulfate, and selenium oxide were used as catalyzers for measuring protein. Catalyzer increases the speed of reaction. It should benoted thatinsample taking, as the amount of sample's proteinincreases, the amountofsample taking for test decreases. Proteinis calculated from below equation:

(1) $$\%\ \text{protein} = \frac{(V_2 - V_1) \times 0/014 \times \text{Protein coefficient} \times N}{\text{sample weight}}$$

To measure the ash,after drying the bush, cool them in desiccator and weigh, then the sample within it is weighed. After that, we place them over the flame, so that the samples are well burnt in a way that white smoke comes out. When the sample is well burnt and its smoking is finished, we take the bushes and place them in furnace for 12 to 24 hours, depending on the type of sample, in order to achieve white gray color. If this color is not achieved, after becoming cold of bush, we add some drops of distilled water and the ash percent is obtained by following equation:

(2) $$\text{Ash percent} = \frac{\text{bush weight and ashed sample} - \text{bush weight} \times 100}{\text{sample weight}}$$

In addition, the evaluation of the amount of fat and pH was addressed. In order to determine the synthesis of amino acids, it was used chromatography. First, the samples were fully hydrolyzed at 110 $^{o}$C for 24 hours by using normal hydrochloric acid 6. Then, using dialdehydeافتال the derivation of amino acid was conducted. The value of total amino acids was done by liquid chromatographywith high performance of kenoer model (Germany) and using spherical column and florescent detector (Antonie F.R., WeiC.I.,Littell R.C., Marshall M.R1999).
Finally, to analyze the obtained data, we used SPSS and one-way ANOVA and for comparison of averages, Duncan comparing method in statistical level of 5% was conducted.

## RESULTS

The results indicated that skin has more amount of humidity, but from the statistical perspective, there is no significant difference between the humidity of skin and fin ($P>0.05$), while the bone contains more fat and ash comparing the fin and skin ($P<0.05$). The results of one-way ANOVA showed that the skin of longtail tunahave higher amount of hydroxyprolineregarding the fin and statistically there is a significant difference between the skin and fin of tuna ($P<0.05$).

Table1. Approximate analysis and contents of hydroxyprolineof skin and fin ofThunnustonggol and extracted collagen of each part

| Sample | The percent of proximate compounds on the basis of wet weight | | | | hydroxyproline(mg/g sample( |
|---|---|---|---|---|---|
| | Humidity | Ash | Fat | protein | |
| Skin | ± 64/08 n.s 0/05 | ± 3/23 c 1/41 | 0/98 ± n.s 0/23 | ± 32/0 a 0/19 | 0/41 ± 19/5 a |
| Fin | 17/63 12/0 n.s | ± 7/32 b 1/07 | 1/19 ± n.s 0/16 | 16/11 ± b 0/14 | 0/22 ± 12/5 b |
| Skin collagen | 0/58 ± 7/06 | ± 0/68 0/09 | 0/33 ± 0/07 | ± 0/94 0/75 | 0/85 ± 58/5 a |
| Fin Collagen | 0/32 ± 5/14 | ± 0/52 0/06 | ± 0/25 0/03 | ± 86/02 0/26 | 0/41 ± 49/05 b |

Different letters of each row show significant difference in Duncan test (P<0.05).

The synthesis of amino acids of skin and fin of the tuna has been shown in diagrams 1, 2 and 3 as the amount of amino acid in 1000 parts of sediment. The results indicated that glycine amino acid (Gly) is existed in all three parts as dominant amino acid. After that, there are the amino acids of alanine, proline, glutamic acid and hydroxyproline. The comparison of collagens in three treatments indicates that skin collagen has more amounts of hydroxyproline, proline, and arginine, but less amount of glycine and hydroxy lysine. Totally, according to table 1, the skin of longtail tunahas higher amount of collagen comparing fin and statistically it has significant difference with other two parts (P<0.05).

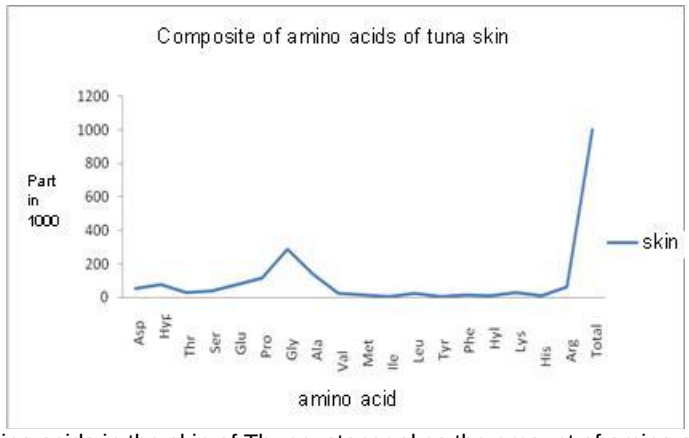


Figure1. The synthesis of amino acids in the skin of Thunnustonggol as the amount of amino acid in 1000 parts of sediment

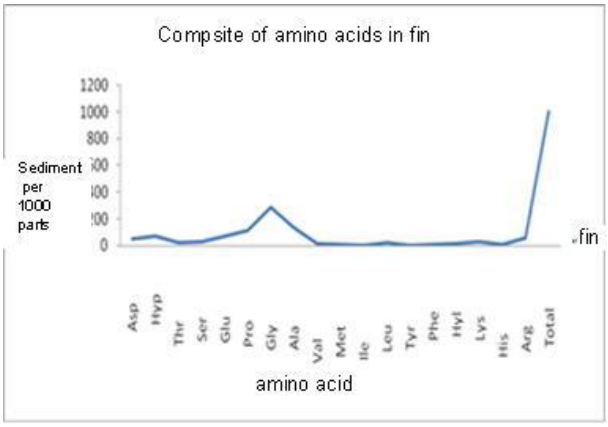


Figure2. The synthesis of amino acids in the fin of Thunnustonggol as the amount of amino acid in 1000 parts of sediment

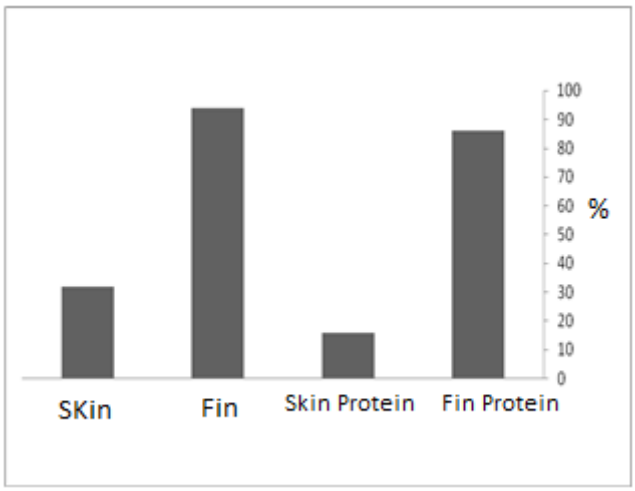


Figure3. Comparison diagram of wastes protein of Thunnustonggol and their collagen

Electrophoresis model of skin and fin under the reduction and non-reduction conditions is shown in figure 1. There is no significant difference in models between collagens of skin and fin. Both collagens include at least two different chains of α including α 1 and α2. Statistically, there was no significant difference between 3 treatments of experiment (P>0.05).

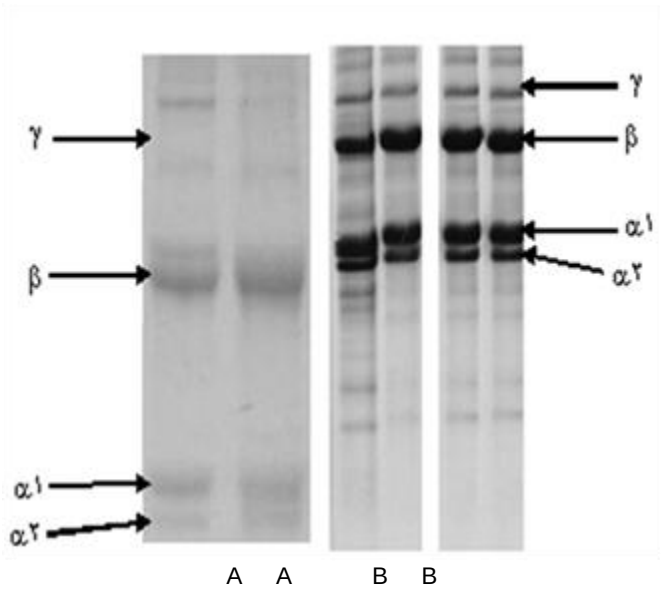


Figure4. Electrophoresis model of skin collagen (A), fin (B) of longtail tunaThunnustonggol

## DISCUSSION

The difference in hydroxyprolineamounts between two species may depend on type of species, environment temperature and the fish body (Shahidi, 1994; Bailey et al., 1989; Morrissey et al., 2000). In this paper when collagen is extracted of skin, the point is that the amount of obtained collagen of longtail tunaskin is more (10.9%) comparing to amount of fin (7.5%). The amount of extracted collagen is related to the content of hydroxyprolineof skin and fin. Sadowska et al. (2003) have reported that hydroxyprolineofcod fish skin was 6.14 mg/g in each sample which was lower than hydroxyprolineof longtail tunaachieved in this study (19.5 mg/g). The extracted collagen of skin and fin of the three has few amounts of ash and fat which indicated the effect of organic substance and fat removal during the collagen extraction operation. The samples of achieved collagen had low humidity and their protein content was varied 84.2% for skin and 86.02% for fin. Generally, the increasing of hydroxyprolinein collagen samples is due to the increasing of their protein content.

Glycine content of skin and fin collagen was approximately 30% of total amino acids. In general, existing glycine allocates near the one-third of total sediments, the amounts of collagen imino acids (proline and hydroxyproline) of skin were 193 and 187, respectively in 1000 parts of sediment that islower respecting to collagen imino acids of mammal skin, like pig (Ikoma et al., 2003). Foegeding et al. (1996) reported that fish collagen has lower imino acids than mammals' collagen and imino acids of animal's collagen relate to their settlement (Foegeding et al, 1996; Rigby, 1968). According to mentioned paper, the degree of hydroxylation proline in collagen of skin and fine are 39.9%, 37.3% and 41.1%, respectively. The degree of hydroxylation lysine in collagen of skin and fin will be 24.4%, 23.6% and 44.4%.Proline and lysine oxidation lead to hydroxy compounds by enzyme of proline hydroxylase and lysine hydroxylase (Burghagen, 1999; Foegeding et al. 1996; Pearson &Young, 1989; Wong, 1989). Previous studies have shown that proline and lysine hydroxylation of bone collagen is slower than skin collagen. Hydroxylationedproline plays the role of triple helix supporter and hydroxylationed lysine participates in formation and maintaining of latitudinal bonds for synthesis with non-hydroxylizedbonds (Ramachandran, 1988; Ciarlo et al., 1997; Kimura, 1985; Kimura and Ohn, 1987; Matsui et al., 1991; Nagai and Suzuki , 2000).

The models of collagen electrophoresis of skin and fin were in reduction and similar non-reduction conditionsand did not show presence of disulfide bands. This result is in line with reported result of hake and salmon collagen (Montero et al., 1990). In general, collagen type I includes less amount of cysteine (less than 0.2%) and methionine (1.24-1.33%) (Owusu – Apenten, 2002) which plays a crucial role in the formation of disulfide bonds. Of course, collagens type III and VI include some cysteine able to be oxidized (Foegeding et al,, 1996). According to conducted analyses on electrophoresis models and constitutive unit's compounds, it can be suggested that the collagen of skin and fin of tuna is collagen type I. This kind of collagen has two certain chains of α 1and α 2 (Burghagen, 1999; Foegeding et al., 1996; Pearson & Young, 1989; Wong, 1989). The studies of researchers have shown that major part of skin and fin collagen is of collagentype I (Ciarlo et al., 1997; Kimura and Ohno, 1987; Montero et al. 1990; Nagai & Suzuki, 2000). It can be concluded that all three types of collagens are rich in internal and middle molecules, the condition of cross linking and also β and γ compounds. The fishes which have suffered hunger or are kept under hunger condition, have more collagen with one bigger degree in cross linking comparing to fishes which are well feed (Foegeding et al. 1996; Regenstein, 1991).

According to findings of current study, it can be concluded that in longtail tunaThunnustonggol, the most amount of humidity and protein is existed in fish skin. Evaluation on skin and fin of tuna indicated that dominant amino acid in all three parts is glycine amino acid and also the amount of hydroxyprolineof skin is more than all other parts which expressed that skin of longtail tunahas the highest amount of collagen and collagen in skin and fin has at least two chains of α 1 and α 2.

## ACKNOWLEDGEMENT

We gratefully acknowledge and appreciate the cooperation and sympathetic assistance of officials of fishery products of Bandarabbass andPersian Gulf and Oman Sea Ecological Research Center.

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