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# Isolation of collagen from some fishes skins in Iran

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Collagen is the most abundant protein of animal origin, comprising approximately 30% of total animal protein. Collagen isolated from limed split wastes can be a high value product due to its special characteristics and has many potential future applications in biomaterials, Food, functional additives, cosmetics and pharmaceutical industries. Extraction of collagen was done in acids and sodium hydroxide different concentrations. Yield of extraction of collagen from *Narrow barred* skin was shown high in comparison with *Carcharhinus leucas* skin. High yield depends on kind sample and the value of collagen exist in Narrow barred skin that it was shown high in comparison with value of collagen of *Carcharhinus leucas* skin. Yield of extraction from *Siganus sutor* skin was shown high in comparison with skin of *Indo Pacific king mackerel*. The high yield depends on kind sample and amount of collagen of skin of *Indo Pacific king mackerel* that it was shown high in comparison with amount of collagen of skin of *Siganus sutor*. Yield of extraction from *Narrow barred* skin was shown high in comparison with others samples. The high yield depends on high concentration of used chemical compounds and kind fish. Therefore, extraction of collagen from skins of fishes of *Siganus sutor*, *Narrow barred* and *Indo Pacific king mackerel* obtained from Persian Gulf of Iran was shown to be suitable from standpoint of economic value.

**Key words:** collagen, fishes skins, extraction.yield.

## Introduction

Collagen is a fibrous protein found ubiquitously in all multicellular animals. It is a particularly rigid and inextensible extracellular matrix protein that serves as a major constituent of many connective tissues. The characteristic feature of a typical collagen molecule, tropo collagen, is its long, stiff, triple-stranded helix, in which three collagen polypeptide chains are wounded around one another in form of a ropelike super helix. These peptides are extremely rich in proline and glycine, both of which are important for the formation of the collagen-specific helical structure (Rossler *et al*., 1995; Mizuta, *et al*., 2004). Collagen plays an important role in the formation of tissues and organs and is involved in various

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cells in terms of their functional expression. Recently alongside clarification of the biological functions of collagen as an extracellular matrix protein, collagen has been attracting attention as a biomaterial with many unique characteristics such as high tensile strength, low antigenicity, bioresorbability, good biocompatibility, induces coagulation of blood platelets, effects cell differentiation, effects wound healing, control of various characteristics through physical and chemical modifications, moldability, abundant and easily purified (Ottani, *et al.,* 2002). Though the invertebrates comprise approximately 95% of the Animal Kingdom, the information about their collagens and extracellular matrices is scarce. The relative complexity of the invertebrate collagens and the difficulty in their purification and characterization has hindered continued progress in their research. Preliminary studies on invertebrate collagens have been reviewed (Skrodzki *et al*., 2003). Added effort on the analysis of invertebrate connective tissues over two decades has led to the identification of genetically distinct collagen types in a number of species and there has been appreciable success in the isolation and purification of single molecular species of collagen.

Fish Collagen is a complex, structural protein that helps to maintain the strength and flexibility of skin, ligaments, bones, joints, muscles, tendons, gums, teeth, eyes, blood vessels, nails and hair (Birk, 1984). Food grade fish collagen is abstracted from fish scales and fish skins, simulating the mechanism of protein digestion in human body and using advanced directed enzymolysis biotechnology. Its protein content is above 90%, having 18 kinds amino acids, 7 kinds of which are essential for people. These products are of high protein content, low ash and heavy metal content, small molecular weight, easy absorption and utilization, high biological value, promoting absorption of vitamins and minerals. These products also can accommodate physiological function of human as directed enzymolysis technology releases a lot of biologic peptides hidden in big molecular collagen(Kolodziejska *et al*., 1999).

## Materials and methods

Fresh fishes skins (Length: 24 cm$\pm$ 6 cm, width: 13 cm$\pm$ 4 cm) were obtained from a local fish shop in Ahvaz, Iran. They were immediately frozen and stored at -18 °C until use. All reagents were of analytical grade.

### *Cleaning of fish skins*

Thawed skins, all coming from the same batch, were washed with tap water (1:6 w/v) in a Stephan homogenizer (position II, very vigorous stirring) (Model UMS; Stephan und Sohne GmbH & Co., Hameln, Germany) at 5°C for

10 min. and were rinsed with abundant running tap eater. Skins were further cleaned with 0.8 M NaCl (1:6 w/v), again in the Stephan homogenizer at 5° C for 10 min, and were rinsed with abundant running tap water. This step was repeated 3 times. Excess water was removed by draining the cleaned skins and manual squeezing.

***Extraction of collagen with the various acids and sodium hydroxide***

Cleaned skins were constantly and slowly stirred for 16 to 18 h at 20°C, with different solutions of acetic acid 1.5, 2, 3, 3.5, and 4% and sulfuric acid of 1, 1.5, 2, 2.5, 3, and 3.5%. The mixture with the remains of the skins was then filtered in a Buchner funnel with Whatman no. 4 (Maidenstone, England) filter paper, and the clear filtrate was then air-dried in a convection oven at 40° C, in the form of very thin layers, until moisture was less than 15%. Extraction with each acid were also done after a pretreatment of skins with sodium hydroxide solutions of 1, 1.5, 2, 2.5, 3, and 3.5% at 5°C for 40 min with constant stirring and rinsing with abundant running tap water (this washing cycle was repeated 3 times). Extraction of collagen was done in different concentrations of acids and sodium hydroxide solutions (Nagai, and Suzuki, 2000; Nagai, *et al*., 2001; Amit Kumar, 2008).

Statistical methods applied in this survey was variance analysis and mean comparison in α- level = 5% through Dancan test, that was performed on qualitative results. Amounts of extracted Collagen from skins of different fishes in acids and alkaline different conditions has been showed in tables of 1, 2, 3 and 4.

**Table 1.** Yield of extraction (%) of collagen from *Siganus sutor* skin.

| **NaoH solution** | **$H_2SO_4$ solution** | **$CH_3CooH$ solution** | **Yield** |
|---|---|---|---|
| 1.5% | 1.5% | 2% | 13.3± 0.78 |
| 1% | 1% | 1.5% | 12.4± 0.86 |

**Table 2**. Yield of extraction of (%) collagen from *Narrow barred* skin.

| NaoH solution | $H_2SO_4$ solution | $CH_3CooH$ solution | Yield |
|---|---|---|---|
| 3.5% | 3.5% | 4% | 16.4± 0.21 |
| 3% | 3% | 3.5% | 15.2± 0.94 |

**Table 3.** Yield of extraction of (%) collagen from *Carcharhinus leucas* skin.

| NaoH solution | $H_2SO_4$ solution | $CH_3CooH$ solution | Yield |
|---|---|---|---|
| 3% | 3% | 3.5% | 14.8$\pm$ 0.47 |
| 2.5% | 2.5% | 3% | 13.9$\pm$ 1.1 |

**Table 4.** Yield of extraction (%) of collagen from *Indo pacific king mackerel* skin.

| NaoH solution | $H_2SO_4$ solution | $CH_3CooH$ solution | Yield |
|---|---|---|---|
| 2% | 2% | 3% | 11.4$\pm$ 1.66 |
| 1.5% | 1.5% | 2% | 10.8$\pm$ 1.09 |

## Discussion

Table 1. showed that yield of collagen extraction from skin of *Siganus Sutor* with $H_2SO_4$ and $CH_3CooH$ various acids at 3.5 and 4% concentrations, including a pretreatment of skins with 3.5% NaoH was shown high. This depends on high concentration of used chemical compounds. Comparison of Tables 2 and 3 showed that yield of collagen extraction from skin of *Narrow barred* in the same chemical condition with H2SO4 and $CH_3CooH$ various acids at 3% and 3.5% concentrations, including a pretreatment of skins with 3% NaoH was shown high in comparison with skin of *Carcharhinus leucas*. The yield of high depends on kind sample and amount of collagen exist in skin of *Narro barred* that it was high in comparison with amount of collagen in skin of *Carcharhinus leuca*. Comparison of Tables 1 and 4 showed that yield of collagen extraction from skin of *Siganus Sutor* in the same chemical condition with $H_2SO_4$ and $CH_3CooH$ various acids at1.5 and 2% concentrations, including a pretreatment of skins with1 5% NaoH was shown high in comparison with skin of *Indo Pacific king mackerel*. This yield of high depends on kind sample and amount of collagen in skin of *Indo Pacific king mackerel* that it was high in comparison with amount of collagen in skin of *Siganus Sutor*. Yield of collagen extraction from skin of *Narrow barred* was shown high in comparison with others samples this yield of high depends on high concentrations of used chemical compounds and variety of fish.

Obtained results from others researchers showed that the collagen content in the skin of Baltic cod (*Gadus morhua*) was 21.5% on the wet weight basis and about 71.2% on the dry weight basis and these results in comparison with our study results was high (Sadowska *et al.,* 2003). Both the collagen and non-collagen protein content in cod skins depends upon the fishing season. Studied and established that the gross composition of skin of cod caught in the same season in different years is almost constant and the collagen content in the skin amounts an average to 21.5% in the wet weight and 71.2% in the dry weight. It was also absorbed that the share of collagen in total protein was considerably determined on the basis of hydroxyproline in samples. Further in the skin of cod the non-collagen proteins, peptides and amino acids were estimated as 4.9% and 16.3% respectively on a wet and dry weight basis. Some studies on collagen reveals that the collagen represents the chief structural protein accounting for approximately 30% of all vertebrate body protein (Liu *et al.*, 2007; Wang *et al.,* 2008; Muyonga *et al.,* 2004). This study can conclude that extraction of collagen from skin of *Siganus Sutor*, *Narrow barred* and *Indo Pacific king mackerel* was shown to be suitable from standpoint of economic value.

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