INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online www.irjponline.com
ISSN 2230 – 8407
Research Article

EXTRACTION AND DETERMINATION OF COLLAGEN PEPTIDE AND ITS CLINICAL IMPORTANCE FROM TILAPIA FISH SCALES (*OREOCHROMIS NILOTICUS*)

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Article Received on: 17/08/11 Revised on: 20/09/11 Approved for publication: 13/10/11

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ABSTRACT
Tilapia are one of the most widely introduced fish globally that has clearly emerged as a very promising group in aquaculture. Oreochromis mossambicus was the first tilapia species to be taken up for large scale aquaculture, followed by Oreochromis niloticus, Oreochromis aureus and Tilapia rendalli. Today O. niloticus contributes more than 80% of tilapia aquaculture production globally. O. mossambicus was introduced in India as early as 1952 with a view to filling some unoccupied ecological niches, mainly pond aquaculture and reservoir fisheries. Tilapia fish scales found to have more percentage of Type I collagen. Fish collagen is complex structural protein that helps to maintain the strength and flexibility of skin, ligaments, bones, joints, muscles, tendons, gums, eyes, blood vessels, nails and hair. Collagen can be obtained from fish scales, fish skins by advanced enzymatic digestion methods in biotechnology. The Protein content of the above is more than 90% and having 18 kinds of amino acids out of which 7 are essential for human consumption. These products are easily absorbable and having high biological value promoting the absorption of vitamins and minerals. The enzymatic digestion technology process releases a lot of peptides in molecular collagen that helps humans in many physiological functions. Collagen has been used in biomedical pharmaceutical, food and cosmetic industries. In this paper we have determined the collagen extracted from Tilapia fish scales and also its clinical importance.

KEY WORDS: Collagen, Oreochromis niloticus, Tilapia, IR spectrum, SDS PAGE analysis.

INRODUCTION

Fish collagen is complex structural protein that helps to maintain the strength and flexibility of skin, ligaments, bones, joints, muscles, tendons, gums, eyes, blood vessels, nails and hair. Collagen can be obtained from fish scales, fish skins by advanced enzymatic digestion methods in biotechnology. The Protein content of the above is more than 90% and having 18 kinds of amino acids out of which 7 are essential for human consumption. These products are easily absorbable and having high biological value promoting the absorption of vitamins and minerals[1,2]. The enzymatic digestion technology process releases a lot of peptides in molecular collagen that helps humans in many physiological functions. Collagen has been used in biomedical pharmaceutical, food and cosmetic industries[2,3]

Most commercial collagens from bovine skin, pig skin or chickens waste. These land animal sources are unsuitable for many religious and ethnic groups, face regulatory and quality control difficulties and can contain biological contaminants and poisons. Such as mad cow disease, foot and mouth disease etc. Thus there is necessity to search for new sources of collagen originating from fish and other sea foods.[4,5]

Collagen peptide extracted from fish scales consisting of small peptide molecules. Its absorption in the small intestine is superior to other collagen products due to smaller molecular size and leads to more efficient collagen synthesis in different parts of the body such as joint tissue, bone, blood vessels and skin dermis. Consequently, this product is used for supplements to lessen the pains and aches due to arthritis, artherio sclerosis and other signs of aging[6,7]. It is widely used in cosmetic to support a smooth radiant, elastic and well moisturized skin to slow down the wrinkle formation. Compared to other collagens from animals like pigs and cattle. Fish collagen is considered as safe.

In the extraction process of fish scale collagen other commercially viable products like gelatin, amino acids and minerals can be obtained. Fish scales are a potential source to extract collagen.

METHODS AND MATERIALS

Method of extraction

Desalting process was carried out first was carried out without using EDTA. Fish scales was soaked in 0.1 M NaOH with a sample ratio of 1:30 (w/v) in order to remove non collagen proteins. Later digestion was carried out at PH 5.6-6.2 at low temperature for 24 hr continuous stirring. After the digestion salting out process is carried out to precipitate collagen and later it is purified by further steps.[1,3,8]

Electrophoresis (SDS-PAGE) experiment was conducted to separate Tilapia fish scale collagen and gelatine protein according to the molecular size, to investigate subunit compositions, verify homogeneity of protein samples, and purify proteins for use in further applications.[9,10]

In polyacrylamide gel electrophoresis, proteins migrate in response to an electrical field through pores in a polyacrylamide gel matrix; pore size decreases with increasing acryl amide concentration. The combination of pore size and protein charge, size, and shape determines the migration rate of the protein.

Method Referred

Laemmle's method for discontinuous gel electrophoresis under denaturing conditions, i.e., in the presence of sodium dodecyl sulphate (SDS).

Basic Protocol: Denaturing (SDS) Discontinuous Gel Electrophoresis

Materials used

Samples (collagen and gelatine) were analyzed for protein. Protein Estimation conducted using quartz cuvettes by UV method at absorbance 280nm (DU 700Spectrophotometer; Beckman Coulter).BSA standard prepared from stock solution of 1mg/ml.Gelatine sample weighed and 1mg/ml of stock prepared. With brief idea of protein concentration samples loaded for SDS-PAGE. 10µl loaded for silver stained gels and 20 µl with Coomassie blue.1X SDS sample buffer used for sample dilution. Protein molecular-weight-standards mixture (High (25000-250,000 Da) and low range (14,000- 97,000 Da) (Biorad's) 10%, 8% and 6.5% of resolving Polyacrylamide gel used precast (Biorad's).

1× SDS electrophoresis buffer. Electrophoresis apparatus (Biorad's), small format with 100-mA capability constant-current power supply (allowing running of two gels simultaneously)

Testing for Anti-aging property of collagen

We have tested on healthy volunteer of age 58 with wrinkles on her head.

SUMMARY OF RESULTS

Protein estimation resulted good amount of protein in both the samples (see TABLE-1,2). In SDS-PAGE protein separation profile is clear, a band of collagen approx >80KDa exhibited in 6.5 % and 8% resolving gels, whereas in 10% it was unclear.Fig-1,2.

A very fine band of collagen protein is also there (See 8% and 10% coomassie stained gel Fig-3,4, which may be a dimer or monomer part of protein.

Comparing the wrinkles before and after the treatment has shown the collagen has got cosmetic importance as well clinical importance. Fig-6,7,8

I.R Spectroscopic analysis of Tilapia fish scale collagen

KBr IR spectroscopic results has confirmed the type I collagen from Tilapia fish scale hydrosylated collagen extract.

The results of IR Spectroscopy indicate that the ionic liquid cross linked biomaterials retained the triple helical structure of tilapia scale collagen and the characteristic Amide II (N-H) bending vibrations were observed at around 1550 cm^{-1}. Similarly Amide I (C=O stretching) and Amide A (N-H stretching) signatures were also seen respectively at 1632-1664 and 3318-3350 cm^{-1}.This shift may stem from coordinate or H-bonding interactions of the amide nitrogen, hence in the present case where all of the anions are capable of multiple H- bonds, it seems likely that this is the origin of the cross-linking effect. Fig- 5

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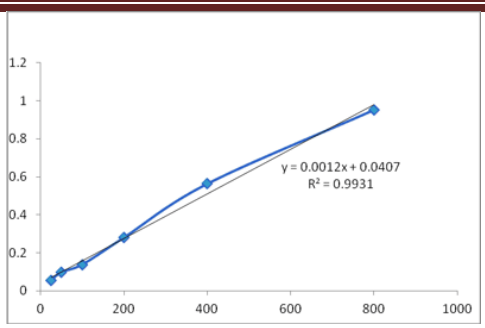
Table 1

std. (BSA 1mg/ml)	Std. Conc.	Abs. at 280nm
1	25	0.055
2	50	0.099
3	100	0.139
4	200	0.281
5	400	0.564
6	800	0.953

Result of protein analysis



Standard Curve: 1

Table 2

Gelatin Sample stock solution: 1mg/ml			
Gelatin Dilutions	Abs.@280	O.D X Dilution Factor	Total Protein (
1:2	1.08	2.16	765.9
1:5	0.934	4.67	662.4
1:10	0.731	7.31	518.4
1:20	0.362	7.24	256.7
Collagen sample diluted from vial			
Collagen Dilutions	Abs.@280	O.D X Dilution Factor	Total Protein (
1:2	2.013	4.026	1427.6
1:5	1.555	7.775	1102.8
1:10	1.033	10.33	732.6
1:20	0.599	11.98	424.8

Total protein calculated using standard concentration and their respected OD. We diluted collagen sample 1:2, which resulted 1.42 mg/ml of protein and loaded 10-20 ml on gel. Gelatine sample loaded on gel from its 1mg/ml of stock solution. (**Note:** Protein estimation we have done for an idea to estimate for sample quantity loaded on gel. Exact protein quantity of both the samples you may know better.)

SDS-PAGE RESULTS

6.5%Resolving

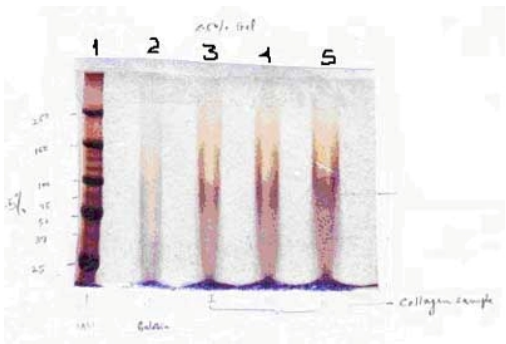


Fig-1 Gel Lane Description: High range precision molecular weight marker, 1.Gelatine 10µl (1mg/ml), 2.Collagen, 3.Collagen, 4.Collagen

8 % RESOLVING GEL

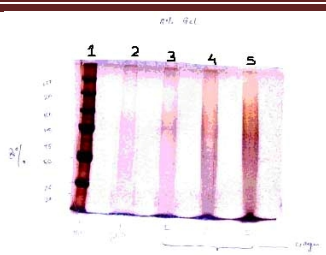


Fig-2 Lane Description: 1.High range precision molecular weight marker, 2. Gelatine, 3.Collagen, 4.Collagen, 5.Collagen

8 % RESOLVING GEL (CBB STAIN)

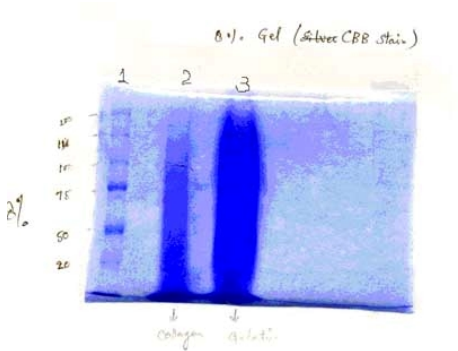


Fig-3, Lane Description : 1.High range precision molecular weight marker, 2.Collagen, 3.Gelatine

10 % RESOLVING GEL

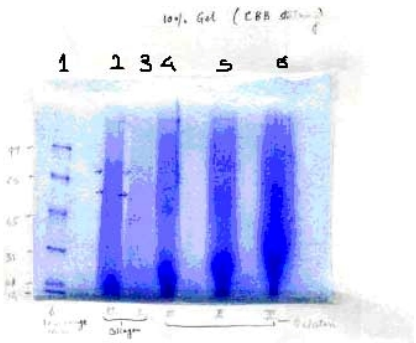


Fig-4, Lane Description, 1.Low range MW (14-97 KDa), 2.Collagen (1:10 diluted), 3.Collagen (1:20 diluted), 4.Gelatine, 5.Gelatine, 6.Gelatine

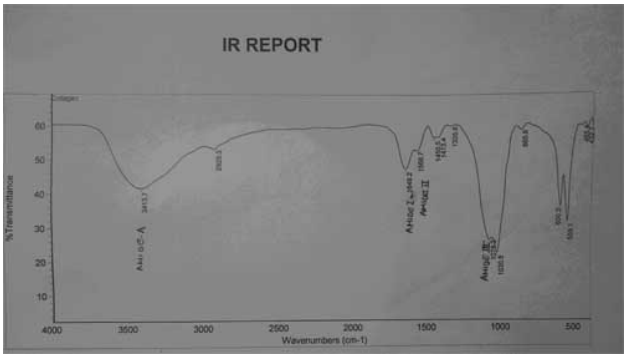


Fig-5, IR Spectroscopy of tilapia fish scale collagen : Showing amide-A , Amide I Amide II Amide III bands confirming the collagen peptide.

Fig-6, BEFFOR TREATMENT

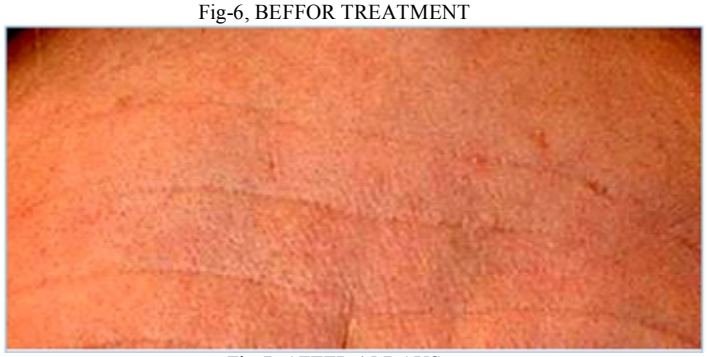


Fig-7, AFTER 15 DAYS

Fig-8, AFTER 25 DAYS OF TREATMENT

Source of support: Nil, Conflict of interest: None Declared