ISOLATION AND DETERMINATION OF TYPE I COLLAGEN FROM TILAPIA *(Oreochromis niloticus)* WASTE

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ABSTRACT:

Tilapia are one of the most widely introduced fish globally that has clearly emerged as a promising group in aquaculture. *Oreochromis niloticus* was the first Tilapia species to be taken up for large Scale aquaculture. It is consumed widely due to its deliciousness and rich source of protein. During its processing, the scales, Fins, Skins etc are expelled out as waste Acid solubilized collagen (ASC) and Pepsin Solubilized collagen (PSC) were extracted from these processing wastes. Initial extraction by acid yielded 22% of collagen and subsequent digestion with pepsin yielded 56% on dry weight basis. The total protein of ASC and PSC was determined by Bradford method which contains 68.34mg/ml,23.24 mg/ml respectively. The FT-IR Spectrum showed that ASC and PSC are helpful in prediction and confirmation of Secondary structure of proteins. The denaturation temperature of ASC was 32°C while for PSC it is 29°C.SEM micrograph showed the fibrous nature of Collagen. This report indicates that Tilapia waste might be useful as a new source of collagen apart from usual bovine and pig skin.

Keywords: *Oreochromis niloticus* processing waste, FT-IR, ASC, PSC

1. INTRODUCTION

Collagen is the most abundant animal protein polymer. About 30% of the total protein in animal body is collagen.It is the major insoluble fibrous protein in the extra cellular matrix and in connective tissues. Generally, collagen has a wide range of application in cosmetics, biomedical, pharmaceutical, leather and food industries[1]. It is also regarded as one of the most useful biomaterials, mainly due to its non-toxicity, biocompatibility, immunological properties, and well documented structure[2]. Type I collagen is present in bones and skin. It represents over 90 percent of total collagen and it composed of two '1 chains' and one '2chain' heteropolymers.

In general, fish muscles and scales contains0.2% to 10% of collagen, these are mainly type 1 collagen. During the processes of Tilapia, a great amount of wastes like scales, fins, skins etc are expelled. These wastes contain a large amount of collagen. Therefore in this paper, we extracted and partially characterized the collagens of Tilapia waste for potential utilization, and as an alternative for pig skin & bovine collage, and as well as it may increase the economic value of Tilapia.

2. MATERIALS AND METHODS

2.1 Sample collection

Tilapia wastes were collected from local fish market in Tiruchirapalli. The skin, scales, and fine were expelled out during processing like cleaning, cutting and filleting. These wastes were collected, cut into small pieces and stored at -4°C until used.

2.2 Extraction of Collagen

The skins, fins and scales used as raw materials were cut into small pieces (0.3x0.3cm) with scissors, soaked with 0.1M NaOH (1:30 w/v) $_{P}$H to remove non-collagenous proteins for 48hours[3,4]

2.3 Acid solubilized collagen

The prepared sample was extracted with 0.5M acetic acid for 42hours at 4-6°C and centrifuged at 30,000x g for 60 min. The supernatant was collected and precipitated by adjusting the pH to around 7, since the iso-electric point of collagen is around 6.8 to 7.2. The obtained precipitate was collected as pellet by centrifugation at 20,000x g for 30 min. The precipitate was dissolved in 0.5M acetic acid, dialyzed against 0.1M acetic acid and deionized water in a dialysis membrane and lyophilized. The dialyzate was referred to as ASC.

2.4 Pepsin solubilized collagen

The prepared sample was extracted with 0.5M acetic acid for 24hrs at 4-6^{o}C and centrifuged at 30,000xg for 60 min. The supernatant was collected and 0.1% pepsin was added and incubated for 6 hrs. It was then precipitated by adjusting the pH to around 7, since the iso-electric point of collagen is around 6.8 to 7.2. The obtained precipitate was collected as pellet by centrifugation at 20,000x g for 30 min. The precipitate was dissolved in 0.5M acetic acid, dialyzed against 0.1 M acetic acid and deionized water in a dialysis membrane and lyophilized. The dialyzate was referred to as PSC.

2.5 Estimation of collagen protein

In Tilapia waste the amount of collagen protein was estimated by Bradford method, with BSA used as standard.[5]

2.6 SDS Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli's [6] using 5% stacking gel and 7.5% resolving gel. The samples were dissolved in 0.6M Tris-Hcl buffer ($_{P}$H6.8) which contained 25%(v/v) glycerin,2%(W/V)SDS,5%(V/V) β-mercaptoethanol and 0.1%(w/v) bromophenol blue. After electrophoresis, gels were visualized with Coomassie Brilliant Blue R-250.

2.7 Fourier Transform-Infra Red Spectrum Analysis

FT-IR spectra of freeze dried collagen samples ASC and PSC of Tilapia was recorded using Biored FT-IR 40 model, USA. 10mg of sample was mixed with 100mg of KBr and was clamped into salt disc of 10 mm diameter for reading spectrum further by using KBr for pelleted forms of Samples. The spectrum of ASC and PSC was recorded, and effective peak was obtained and were assigned with that of standard collagen.

2.8 Morphological Analysis

Morphological analysis was undertaken using a QUANTA-200, S3400Sem (FEI company, USA) at an accelerating voltage of 15KV.The ASC and PSC were coated with platinum for morphological observations.

2.9 Determination of Denaturation Temperature

The denaturation temperature was measured according to the method of Nagai et.al.[7]. Viscosity measurement plays a major role in this determination. 10ml of 0.03% collagen solution in 0.1M acetic acid used for viscosity measurement, and the measurement was done using Ostwald Viscometer. This solution's viscosity was measured at several temperatures from 10^{o}C to 60^{o}C. As the temperature was raised stepwise and maintained for 30 mins at each point, the thermal denaturation curve was obtained by measuring the solution's viscosity at these points. The denaturation temperature (Td) was determined as the temperature at which the change in viscosity was half completed.

3. RESULT

The investigation of this study in the first reporting of isolation and determination of type I collagen from Tilapia (*Oreochromis niloticus*) wastes. The protein estimation resulted good amount of protein in PSC. The yield of ASC was very low compared to PSC. The value of ASC was about 22% based on the lyophilized dry weight, while PSC yielded 60% on the same basis. The obtained result was higher to that of Jelly fish (25-35%)[9] , Baltic cod (21.5%), Japanese sea bass (51.4%), Crassostrea gigas (11%)[8], chub mackerel (49.8%)[9], and Bull head shark waste (50.1%). Thus, this suggests that the process wastes of Tilapia were potential source of alternative natural collagen.

4. DISSCUSSION

4.1 SPS – PAGE analysis

Tilapia collagens were examined by SDS-PAGE. Both ASC and PSC shows to contain at least two different α chains. PSC was comprised of both α1 and α2 chains, but the separation of these chains were not as prominent as in ASC. It was found that these collagens had a chain composition of two α1 chains and a single α2 chain[10]. More amounts of β chains and molecular cross linked components were also found in Tilapia waste collagen. But there are no report found about α3 chain found in fish collagen. These results indicated that ASC and PSC extracted could be characterized as type I collagen with reference to standard acid-soluble type-I calf-skin collagen.[11,12]

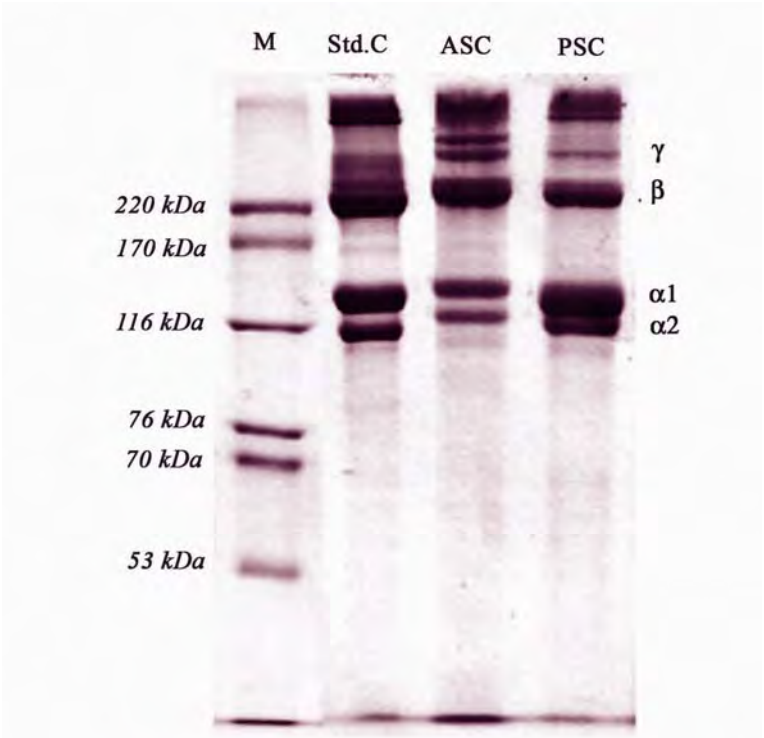


FIGURE -1 SDS - PAGE of Extracted collagen and high molecular weight markers.

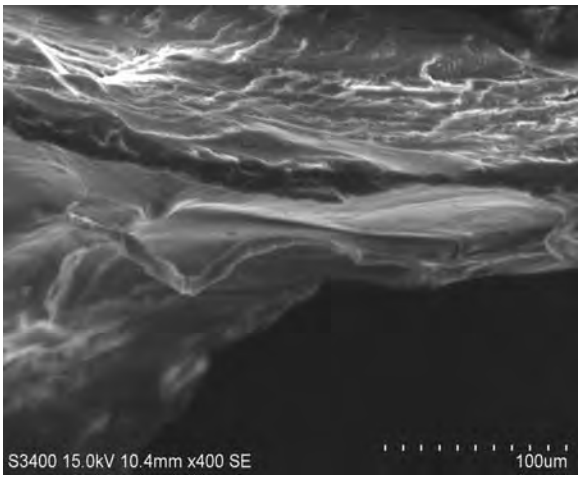
M - Marker
Std.C - Standard Collagen
ASC - Acid Solubilized Collagen
PSC - Pepsin Solubilized Collagen

4.2 **FT-IR analysis**

FT-IR. Spectroscopic results has confirmed the type-I collagen from Tilapia fish waste. The presence of secondary protein structure was assigned with standard collagen spectral range. Amide II (N-H) bending vibration were observed at around 1550 cm^{-1}. Similarly Amide I (C=0 stretching) and Amide A (N-H stretching) signatures were also seen respectively at 1632-1664 and 3318 – 3550 cm^{-1}. Thus, from the spectral data, it was helpful in prediction and confirmed the secondary structure of proteins isolated collagen from Tilapia process wastes.

4.3 Morphological Analysis

The Tilapia process wastes composed of extra cellular matrix, mainly Type I collagen fibers which together formed a highly ordered 3D structure. The regions consists of many layers, mostly fibrillary, and flaky in random orientation, in both ASC and PSC.



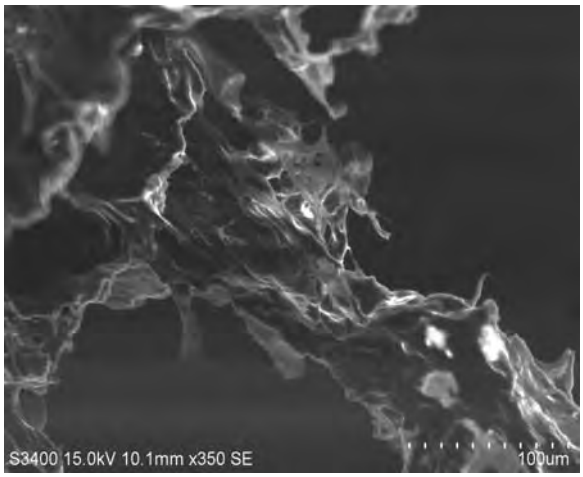


FIGURE 2.and FIGURE 3. SEM micrograph of fibrous layers of Acid Solubilized Collagen (ASC) and pepsin solubilized collagen (PSC) of Tilapia waste

4.4 Denaturation Temperature

The denaturation temperature (Td) of Tilapia wastes was calculated from the thermal denaturation curves **Figure4**and**Figure5.** Td of collagen was taken to be the temperature at which the fractional viscosity was 0.5. Td of ASC of Tilapia process wastes was about 32°C but the Td of PSC of Tilapia process waste was only 29°C, which was mainly due to the hydrolysis action of the enzyme. Td of ASC was lower than the porcine skin collagen, but higher than the Td of many fish collagens measured under the same conditions. [13, 14, 15]

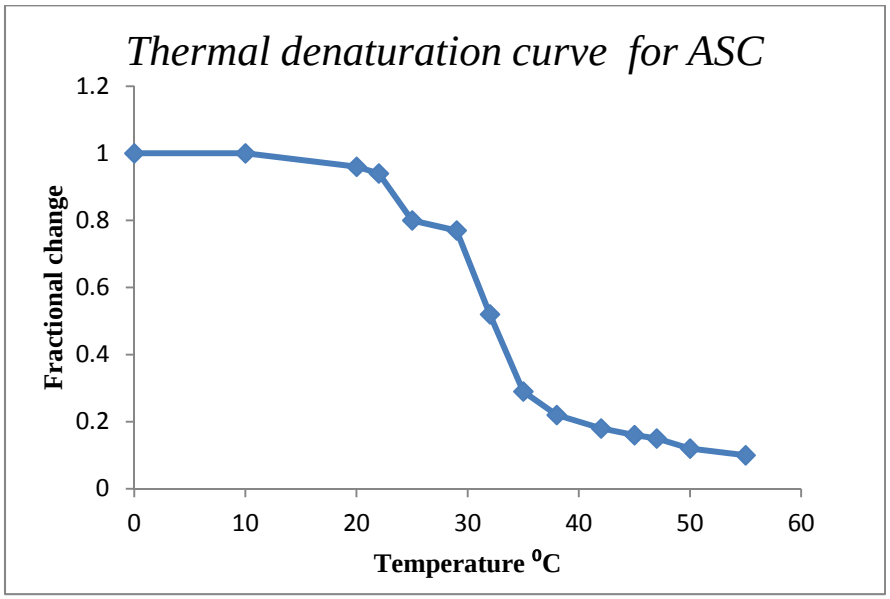


FIGURE 4: Thermal denaturation curve of Tilapia waste collagen solution (ASC) as measured by viscosity in 0.1M acetic acid. The incubation time at Each temperature was 30 min. collagen concentration: 0.03%

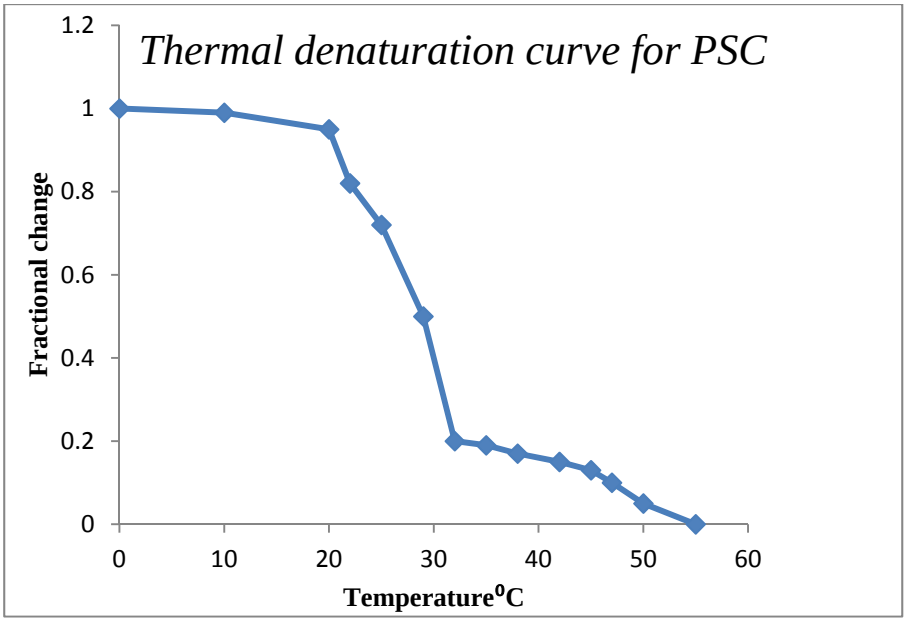


FIGURE 5: Thermal denaturation curve of Tilapia waste collagen solution (PSC) as measured by viscosity in 0.1M acetic acid. The incubation time at Each temperature was 30 min. collagen concentration: 0.03%

5. CONCLUSION

In this study the Tilapia process waste collagens were extracted by 0.5M acetic acid or pepsin. This found to contain higher amount of type I collagen. The SDS-PAGE indicated the presence of this type of collagen in ASC and PSC and it was further confirmed by FT-IR analysis. Among ASC and PSC, β chains were present in the sample level, but α chains were present in higher level in PSC. SEM analysis confirmed the fibrous nature of Collagen and low thermal denaturation temperature was recorded. It was found that a great amount of Tilapia process wastes are expelled, but the results showed that it is possible to use these waste as an important and alternative collagen source.

6. REFERENCES

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