Functional Properties of Collagen Extracted From Catfish (*Silurus triostegus*) Bone

Ayat A. Abbas[1] **K.A. Shakir**[2]

Affiliation: [1] Environmental Dept. Biotechnology Research Center/ Al-Nahrain University
[2]Dept. Food Science Collage of Agriculture / University of Baghdad

* Correspondence: ayatadnan79@gmail.com

Received: 25/5/2022

Accepted :15/8/2022

Published:18/12/2022

Abstract:

Back ground: Collagen accounts for 25percent of all proteins found in vertebrates. The words "collagen" is created with Greek words "kolla" and "genos," which respectively mean "glue" and "formation." It has a wide range of biological and pharmacological uses. Pig and cow skins provide the majority of the collagen. Utilizing animal collagen has been restricted as a result of epidemics of certain animal diseases including foot-and-mouth disease (FMD) and bovine spongiform encephalopathy (BSE) (FMD), which pose a risk of transmission to humans

Objective: The goal of this study is to extract collagen from catfish bone.

Material and methods: Catfish (Silurus triostegus) bone was used to extract collagen that has been pepsinized and acid-solubilized collagen (ASC) (PSC). The extracts' proximate composition (moisture, protein, fat, and ash) was measured, and dry weight was used to compute the yield of ASC and PSC. The molecular weight of experimental collagen ranged from 97 to 200 KD, according to the SDS-PAGE pattern. In order to identify bone collagen, the Fourier Transform Infrared (FTIR) spectra analysis for the acquired ASC and PSC, as well as the standard collagen (type I, II), was used. Using a scanning electron microscope (SEM, the morphological structure of ASC and PSC was revealed), and the diffraction angles (2 θ) of ASC and PSC were determined using X-ray analysis. For quality control, the HPLC technique was used to determine the collagen.

Rustles: The proximate composition of ASC and PSC bone of catfish. The ASC bone of catfish contained moisture (75.22%), good amount of protein (16.65%) , fat (2.40%) and ash content (0.28 %), While the PSC bone had slightly lower moisture (68.50%) than ASC, it had higher protein (19.93%), fat (1.825%), and ash (0.58%) levels ,HPLC SEM ,FTIR ,Electrophoresis, X-ray.

Conclusion: Fish bone was promising sources for preparing collagen, acid soluble collagen (ASC), pepsin soluble collagen (PSC) were successfully extracted by two methods. The collagen extracted from bone catfish, was of type I collagen, which are composed two α1, one α2 chains, and β chains as shown by SDS-PAGE patterns.

Keyword: catfish, bone, HPLC, X-ray, FTIR, SDS, SEM, ASC, PSC.

Introduction:

Collagen accounts for 25% of the total proteins in vertebrates. The words "collagen" is derived from the Greek words "kolla" and "genos," which respectively mean "glue" and "formation." It has a wide range of biological and pharmacological uses. Pig and cow skins provide the majority of the collagen. The use of animal collagen has been restricted as a result of epidemics of certain animal diseases such as bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD), which pose a risk of transmission to humans [1]. Some peoples, their beliefs, refuse to eat pork and its derivatives, as well as cows in other peoples [2]. In such cases, fish collagen is the ideal option due to its high availability, lack of disease transmission risk, and lack of religious obstacles. Fish collagen differs from animal collagen in several ways, including its high biological value, high essential amino acid content, and low hydroxyproline level. By-catch of unutilized and underutilized fish species, as well as fish processing discards, are prospective sources for extracting fish collagen. Skin, bones, scales, and fins are common components of fish processing waste. Fish processing activities in India generate around 2 million metric tons of waste each year [3]. Processing discards from fisheries contribute for up to 70–85 percent of total catch weight [4]. They're either dumped on land or towed into the sea. Seafood processors face environmental issues when it comes to disposing of this waste.

The aim of this work was to describe how to determine the acid soluble and pepsin soluble collagen content profile from the bone of freshwater fish, notably catfish, which is plentiful in the Baghdad market. The literature on this subject is currently quite scarce. Despite the fact that several alternative sources of collagen have been widely researched, there is no information on the direct assessment of collagen concentration from the extraction media. In comparison to the physiochemical and biochemical aspects of extracted collagen, the quantitative investigation of collagen extraction received less attention [5].

Materials and Methods:

The fresh Catfish (*Silurus triostegus*) were acquired from a local Baghdad source and transported to the laboratory in the icebox.

Acid Soluble Collagen

The bones of the fish were separated by a hammer and soaked in distilled water for 5 hours. The bones were submerged in 0.1M NaOH for 24 hours at a solid-to-liquid ratio of 1:5 (w/v) to extract non-collagenous proteins. The alkaline solution was altered every six hours. Up to pH 7.5, the bone samples were washed with distilled water, and then EDTA (0.5 M) solution was added, and left with 10 % butyl alcohol (1:10 w/v) for overnight for 5 days. The de-fatted bone samples treated with alkaline treatment were washed with distilled water thoroughly, then blended at 1:4 ratio (solid: liquid w/v) with 0.5M acetic acid and left for collagen extraction overnight. The obtained extract was filtrated, and the insoluble matter was re-extracted for 2 more days with the same solution at 1: 2.5 (w: v ratio). The filtrate was pooled and salted-out from both extraction steps [6].

Pepsin Soluble Collagen

Extraction of pepsin soluble collagen (PSC): The PSC was obtained by the same method as the ASC with the exception that alkali treated bones were continuously stirred in 5 volumes (v/w) of 0.5 M acetic acid containing pepsin with an enzyme/substrate ratio of 1:40 (w/w) for 20 hours. To chart the growth in pepsin soluble collagen content in the medium over time, 8 ml of the extracting medium were extracted every hour for the first four hours of extraction and then every four hours after that [5].

Proximate composition

Standard methods were used to determine the bone's proximate composition. The hot air oven method was used to determine the moisture content. Kiljdhal digestion system was used for sample digestion and kiljdhal distillation system was used to determine nitrogen content for protein analysis. After that, the crude protein was estimated by multiplying the nitrogen content by 6.25. The crude fat was determined by Soxhlet method using petroleum ether (60–80 °C) as solvent. The ash content was determined by a Muffle furnace (Servo, Salem, India) set at 500–550 °C for 15 h. Total collagen content was determined from the hydroxyproline content multiplied by the factor of 7.75. Hydroxyproline content was estimated following the colorimetric method at 558 nm[7] using a UV–vis Spectrophotometer (Selecta,Spain)

The Yield of Collagen

The yield of ASCs, PSCs were calculated based on the dry weight of pretreated material:

Yield (g/100 g) = (Weight of lyophilized collagen)/(Weight of initial dry fish pretreated byproduct) × 100 [8].

Determination of Total Protein

The protein concentration was measured using the biuret method [9]. Using the UV-VIS-spectrophotometer (Selecta, Spain) at 540 nm.

High pressure liquid chromatography

For chromatographic separation, an HPLC method from Shimadzu was used, applying a flow rate of 0.8 mL/min on each column (250 mm length 4.6 mm i.d., 5.0 µm). For the chromatographic separations, an isocratic gradient with a mobile phase consisting of 150 mM of phosphate buffer, pH= 7.0, was used. At room temperature, the column temperature was preserved. In order to eliminate carry-over contamination, the auto-sampler syringe and the injection valve were successively washed with methanol/water (70/30; v/v). 5 µL was the injected sample volume into the device. For the detection of collagen at a wavelength of 214 nm, a diode array detector of Elite from Shemadzu was used [10].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS –PAGE)

According to [11], SDSPAGE was carried out utilizing a dis-continuous Tris-HCl/glycine buffer system, 7.5 percent resolving gel, and 4% stacking gel. To achieve a final collagen concentration of 1 mg/ml, the collagen sample was dissolved in sample buffer (0.5 M Tris HCl, pH 6.8, 2% SDS, 25% glycerol), 10% ME, and then heated for 3 minutes. The gel was stained for 30 minutes with 0.25% Coomasie Brilliant Blue R 250 solution, and then the bands were removed from the gel using a 7.5% acetic acid/5% methanol solution [12].

X-Ray Diffraction (XRD) Identification determination of the various crystalline compounds known as phases were studied using XRD.

The X-ray Diffraction System Works:

1. The sample can be in the form of a powder, cast, or a thin film, and a small granular size (less than 50 microns) and be crystallized.

2. The model is examined by placing it in the designated location inside the system and when the model is exposed to X-rays [13]. And thus we obtain a diffraction pattern from groups in different positions. .Diagnostic Method: The diagnostic process is made by matching the results of the crystallization clearance (d-spacing) that we obtain from the diffraction pattern and comparing it with the standard X-ray diffraction cards that contains information about standard models by using XRD- 6000 (Shimadzu 220 V /50Hz).

FT-IR determination

FTIR was performed using KBr pellets obtained from discs containing ~200 mg potassium bromide (KBr) 2-mg collagen samples under dry conditions. The SHIMADZU (Japan) model instrument has a wavelength of 400-4000 cm-1 [14].

Scanning Electron Microscope (SEM)

Structure examination by the Scanning Electron Microscope (SEM) of extracted collagen. The sample was gold coated with an automated fine ion coater (JEOL JFC-1600) and the structure was studied with a 20 kV scanning electron microscope (TESCAN,VEGA III) like the voltage accelerating.

Results

The proximate composition of ASC and PSC bone of catfish are given in Table 1. The ASC bone of catfish contained moisture (75.22%), good amount of protein (16.65%), fat (2.40%) and ash content (0.28 %), while the PSC bone contained slightly low moisture (68.50%), higher protein (19.93%) ,fat (1.825%) and higher ash contents (0.58%) than ASC. We observed that higher ash content in the PSC bone catfish was caused by increasing mineralization with aging. But, the higher ash content noticed in the bone of leather jacket could not be attributed to the increased mineralization; due to biochemical composition. Presence of relatively high protein in the bone made them good sources for collagen extraction. The moisture and crude protein contents in the muscle of fish species generally ranged between 54% and 80.3% and 16.1–27.9%, respectively [15]. Such high concentration of protein was not noticed in this species so termed as trash fish of low economic value.

Table (1) Chemical composition for Acid soluble collagen ASC and pepsin soluble collagen PSC.

Sample	Protein%	Moisture %	Fat %	Ash %
Bone ASC	16.65	75.22	2.40	0.28
Bone PSC	19.93	68.50	1.825	0.580

High Performance Liquid Chromatography (HPLC)

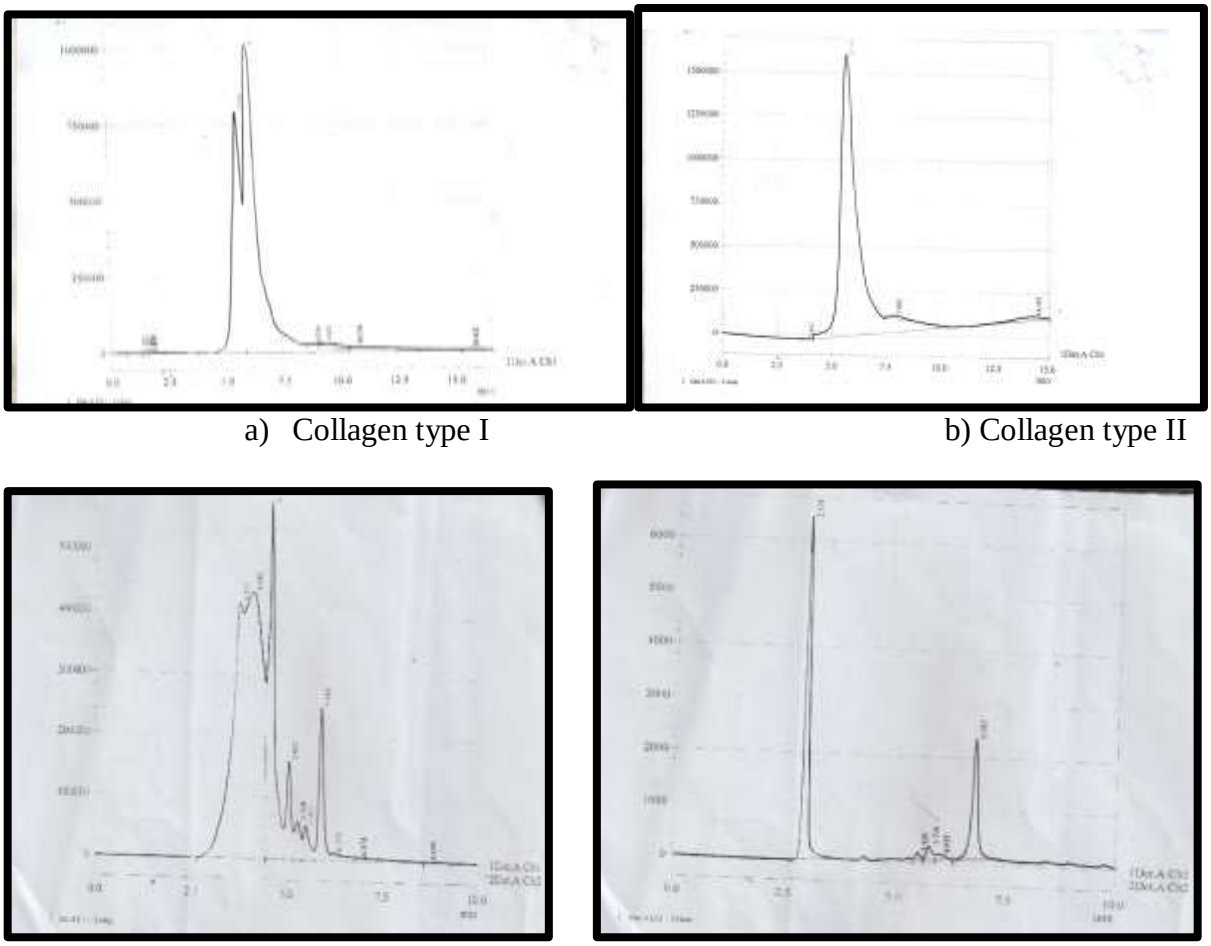
Figure (1 a and b) show peaks for ASC and PSC, retention time and area of standard collagen (I and II). Figure (1 c and d) show peaks, retention time and area of ASC and PSC results obtained from the HPLC UV-VIS analysis. Bone collagen ASC and PSC amount reached to (0.0007-, 0.7810) µg/ml according to the retention time peak collagen type I table (2 a), and bone collagen ASC and PSC according to type II amount was recorded (0.0027 , 0.0317) µg/ml respectively according to the retention time for two peaks collagen type II Table (2b). That's represent the residual collagen in collagen hydrolysate.

Table (2 a): Concentration of collagen in ASC bone collagen and PSC bone collagen according to (I) by HPLC.

Sample	Retention Time (min.)	Concentration (µg/ml)
Standard collagen type (I)	**5.595**	**25**
Bone ASC	**5.881**	0.0007
Bone PSC	**5.519**	**0.7810**

Table (2 b): Concentration of collagen in bone ASC collagen and PSC collagen according to (II) by HPLC.

Sample	Retention Time (min.)	Concentration (µg/ml)
Standard collagen type (II)	**5.564 , 6.055 Two peaks**	**25**
Bone ASC	**5.481**	**0.0027**
Bone PSC	**5.519**	**3.0317**



a) Collagen type I b) Collagen type II

c) ASC d) PSC

Figure (1): High performance liquid chromatography of collagen a) Type I , b) type II c) Bone collagen extracted by ASC ,d) PSC from catfish.

SDS-PAGE

The SDS-PAGE patterns of EDTA-ASC and EDTA-PSC are shown in Figure 2. Two distinct species were observed at different positions and mobility of the α regions of the samples. These patterns indicated that tilapia bone collagens are trimers containing two distinct α chains (α1 and α2) that are similar to those of a typical triple-helix structure of type I collagen with two distinct α chains. This finding indicated that EDTA-ASC and EDTA-PSC are type I collagen.

The molecular weights of α1 and α2 EDTA-ASC and EDTA-PSC subunits were approximately 110 kDa and <100 kDa, respectively, which are different from those of carp bone collagen (α2 is exactly 116 kDa),[16] alligator (Alligator mississippiensis) bone collagen (α1 and α2 chains are 123 and 110 kDa, respectively),[17] backbone collagen of Baltic cod (α1 and α2 chains are visible near 116 kDa), [18] and black drum and sheephead sea bream bone collagen (α1 and α2 chains are 130 and 110 kDa, respectively). [19] Moreover, high molecular weight components were observed in EDTA-ASC and EDTA-PSC, including β and γ components. HCl-PSC contained higher proportions of β components with molecular weights of >200 kDa, peptides with molecular weights (α-chains, whose molecular weights were approximately 100 kDa) of <97 kDa were also found. These results indicated that a greater degradation of αchains was observed in HCl-PSC than in other collagens. Moreover, HCl-PSC was not a collagen similar to gelatin or collagen peptides. The SDS-PAGE pattern of residues in acid solution used in the removing of bone minerals is also shown in Figue 2. Higher proportions, such as β and γ components, were not found. On the contrary, a lot of fragments of peptides were determined. This result indicated that a part of collagen in the bone was dissolved and degraded in HCl solution during de-saluting.

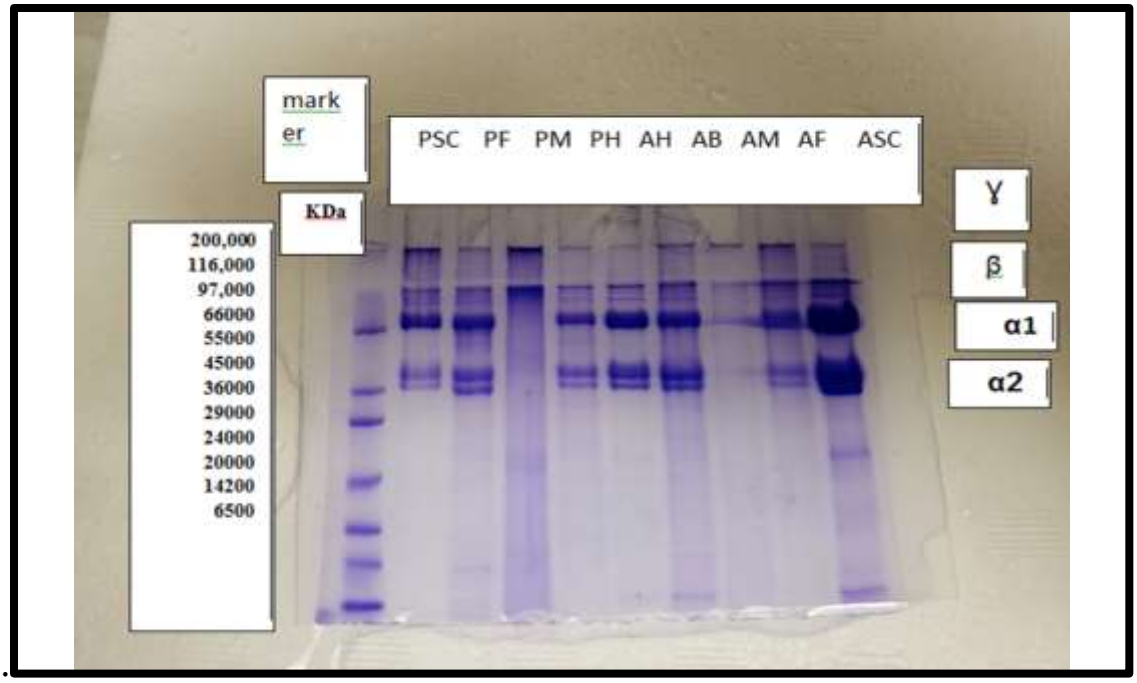


Figure (2) : SDS-PAGE analysis of collagen extracted from catfish wastes (skin PSC ,fin pepsin PF, MP muscle pepsin ,PH head pepsin ,AH head acid, AB bone acid , AM muscle acid , AF fin acid and skin soluble collagen ASC) .

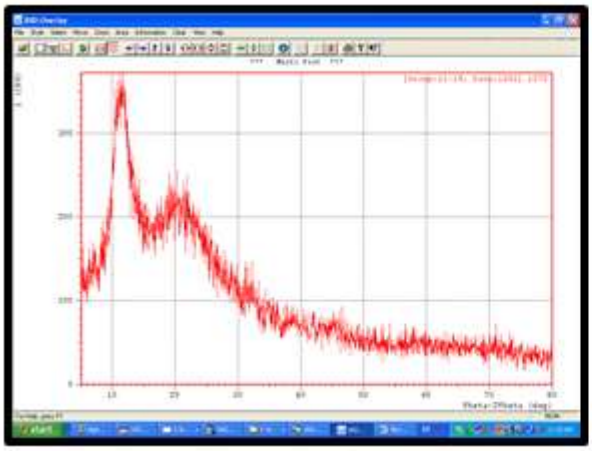
X-Ray Diffraction (XRD):

The aim of experiment to identify the phase of the material crystal or amorphous. This X-ray diffraction of standard collagen (I a, II b) lyophilized acid-soluble collagen ASC c) and pepsin soluble collagen PSC d) from catfish skins are illustrated in Figure (3).

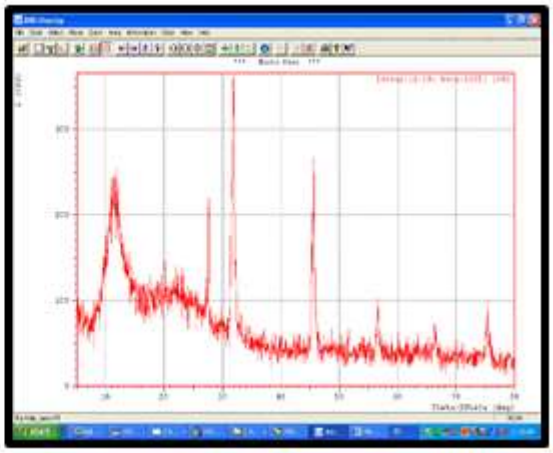
From the devise reading , the diffraction angles (2θ) of ASC were about (12.0178 , 28.0923, 20.7398-)° respectively, the (d) values corresponding to the sharp peak were (7.35841,3.17383,4.279) Å for ASC ,and (2θ) angels for PSC (11.844 ,27.986 , 22.727) ° , and d-spacing (7.466 , 3.186 , 3.909) Å ,compared with the standard collagen type I and II diffraction angles (2θ) were 11.815 ,27.853 ,23.558 ° with d-spacing 7.484 ,3.200 ,3.773 Å respectively ,and for type II angles (2θ) were 11.733 ,12.882 ,13.33° with d-spacing were (7.536, 6.866 ,6.636) Å. These peaks in (2θ) for PSC are more intense and steeper than the peaks observed in ASC .The difference in organization between the structures of ASC and PSC collagen were visible, with ASC collagen spectrum presenting three peaks well featured, meanwhile PSC spectrum presenting three sharp and intense peaks but showed like a flattened pattern.

Crystal structure of acid soluble collagen ASC and a crystal and amorphous for pepsin soluble collagen PSC were showed three sharp diffraction crystal and amorphous peaks located at the diffraction angles, these three peaks are characteristic of collagen molecule and can be seen as amorphous. The sharpest wide peak is related to the triple helix conformation and distance between molecular chains, and for PSC had three peaks crystal and amorphous are related also to the triple helix conformation and distance between molecular chain to the distance between the skeletons. [20] studying collagen prepared from carp scales, he found the minimum d-spacing for the sharp peak (11.87) A^o and (4.48) A^o for the wide peak. From the X-ray diffractograms, it is proven that the morphological characteristics of the two types collagen have

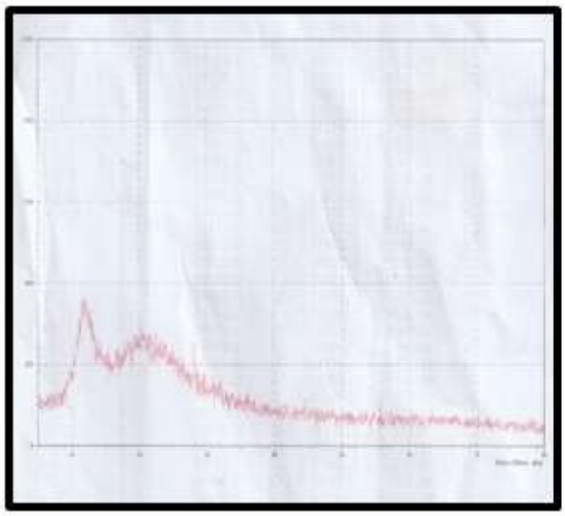
direct relationship with the phase structure of the material , X- ray diffraction is often used to assess collagen fibril distribution and orientation in fish mineralized tissues [21; 22].



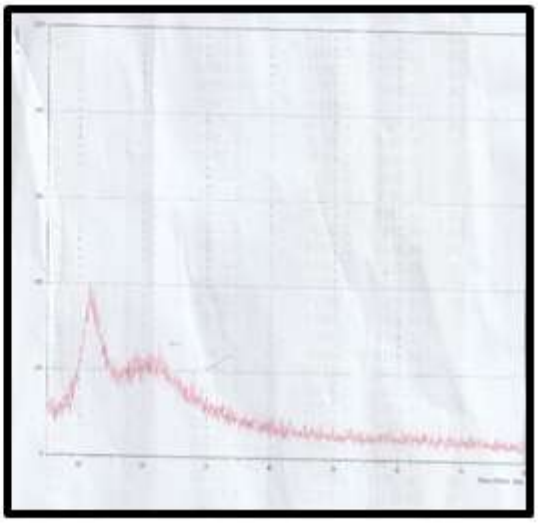
a) Standard(I)



b)Standard II



C) ASC



d) PSC

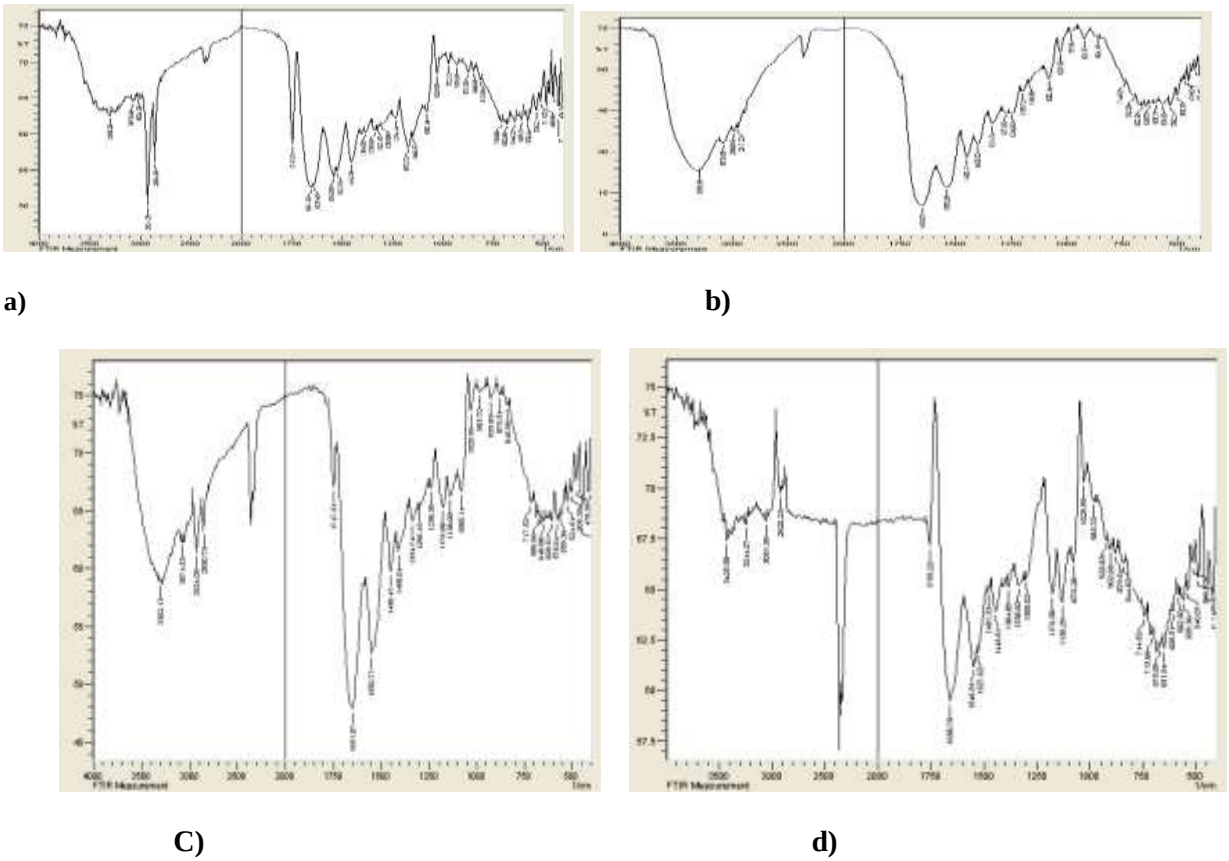
Figure (3):X-ray comparative study pattern of collagen a) standard I, (b) standard II and (c) for ASC, (d) for PSC from the bone of catfish.

FTIR of Bone Catfish

The desalting procedures of tilapia bone were investigated using FTIR. The imges of FTIR of the PO4 3– band, which is seen in hydroxylapatite, were observed at 1030 cm^{-1} in Figure 4. [23] In the infrared spectra of desalinated bones, however, determining the phosphate anion's peaks was challenging. The FTIR method is used to investigate changes in protein structure. Because of the hydrogen-bonded hydroxyl groups, the amide A and B band position in ASC and PSC were 3302.13,3074.53

comparison to type I collagen was 3272 cm^{-1} (Figure. 4b). The amide B band was discovered at 3068 cm^{-1}, which is where it is usually found in collagen. [24].

Amide I was 174151,1662.64 and 1747.51, 1651.07cm1 for ASC and PSC, respectively, in comparison to standard collagen type I and II, which were 1747.51, 1654.92, 1639.49 cm-1 and 1741.51,1651.07 cm-1, respectively, indicating that the collagen fibers are richer in pyridinoline-type cross-linking, proline and/or hydroxyproline, and H-bonding [25].
In compared to conventional collagen type I and II, which were 1543.05, 1523.76,1454.33 cm-1 and 1539.20,1450.32 cm^{-1} respectively, the amide II band associated with N-H stretching vibration was 1550.77, 1450.47, 1411.89 cm^{-1} for ASC and 1550.77, 1450.47, 1408.04 cm^{-1} for PSC. The amide II beam is created by the NH plane's curvature vibration and the CN plane's stretching vibration; it is less impacted by secondary structure, but it is affected by moisture; and the rise in the wavelength of this beam is caused by an increase in humidity in the molecular structure [26].
In comparison to conventional collagen type I and II, the Amide III band associated with coupling N-H via elongation of CH2 1377.17,1342.46, 1284.59,1242.16, 1172.72,1149.57 cm-1 for ASC and 1334.74, 129616,1238.30,1176.58 cm-1 for PSC were 13338.60,1323.17, 1303.88,1234.44 cm-1 and 1334.74, 1273.02,124 In infrared spectroscopy, amide III is a weak beam that returns to the planar bending vibration of the NH group coupled with the stretching vibration of the CN group resulting from amide bonding, in addition to the absorbance resulting from the oscillatory vibration of the CH2 group in the amide bonding [27], In addition to the absorption coming from the oscillatory vibration of the CH2 group in the glycin and proline side chain structure, the beam returns to the planar bending vibration of the NH group coupled with the stretching vibration of the CN group resulting from amide bonding [28] . The secondary structure of the resultant PSC may be changed to some extent when pepsin cleaves the telopeptide region of tropo-collagen [29; 30]. Collagen from the skin of splendid squid exhibited absorptions at 1031, 1060, and 1081 cm−1, which arise from the C-OH stretching vibrations of the carbohydrate moieties attached to the protein [31]. The result suggested that the collagens might consist carbohydrates, which are attached to hydroxylysine residues of the polypeptide chain by O-glycosidic bonds. The result was also in accordance with total sugar content. The result indicated that the triple helical structure might be slightly affected by pepsin digestion during the extraction of collagen.



a) **b)**

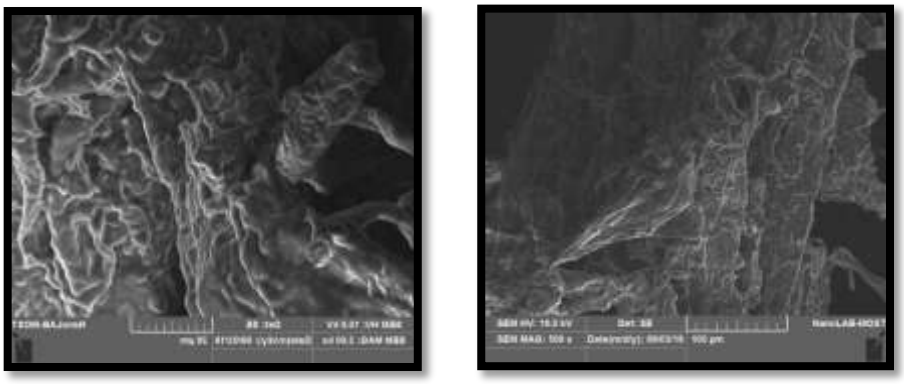
C) **d)**

Figure (4): Fourier Transform Infrared spectra of (a) standard I, (b) standard II and (c) for ASC , (d) for PSC from the bone of catfish .

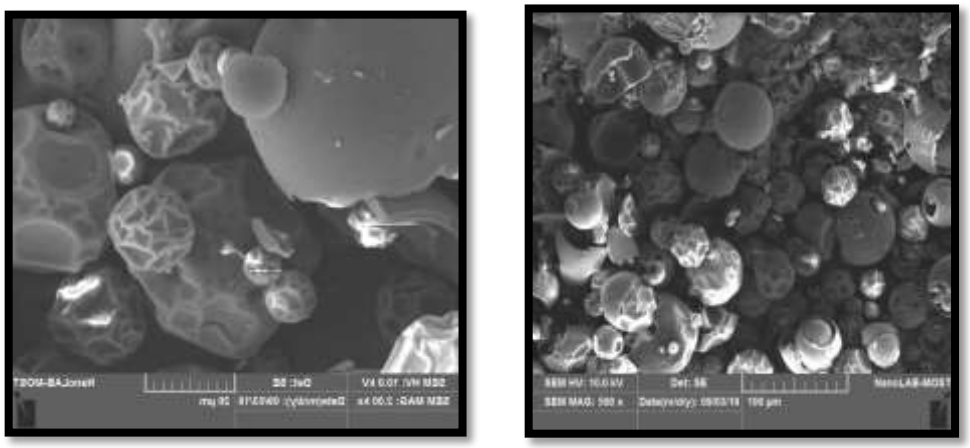
Scanning Electron Microscope (SEM)

The change in structural morphology of fish bone collagen was observed by SEM images in Figure (5) . Morphology of fish bone collagen has a powerful variation among sample, the experimental collagen was observed under naked eye ,and SEM .Under naked eye Figure (5 a , b) show the collagen ASC and PSC appeared as a soft white sponge. SEM analysis for (ASC, PSC ,type I collagen and type II collagen) is illustrates in (Figure5 c, d, e and f) shows the surface morphology of two standard collagen I and II are greatly different from each other

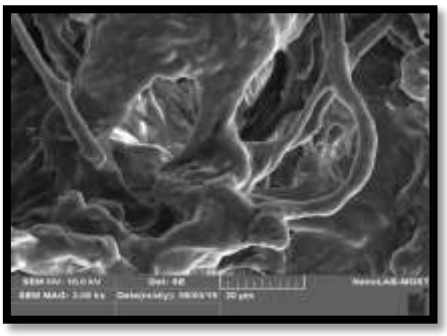
, there are obvious rough surface with globular structure under10 kv, 2kx and 10 kv, 5 kx respectively. The coil-like fibrils were found as a sheet inter-connected for ASC and random windings of coil-like structures for PSC which indicates the fibrous nature of the collagen where seen in Figure (5 e,f) respectively . At high magnification, there is a clear differences between in the microstructure of ASC and PSC, but the collagen of PSC in high magnification appeared regular and uniform network with porous and honey –comb like structure .This is consistent with [32] when he was measured the SEM for ASC and PSC from outer skin of spineless cuttlefish *Sepiella inermis*.

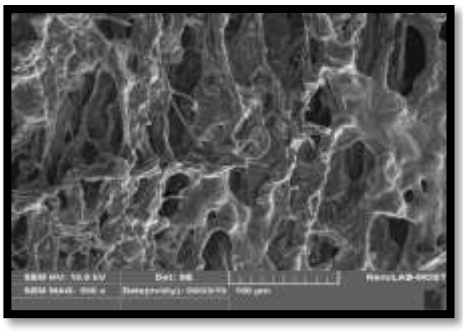


a) St.I

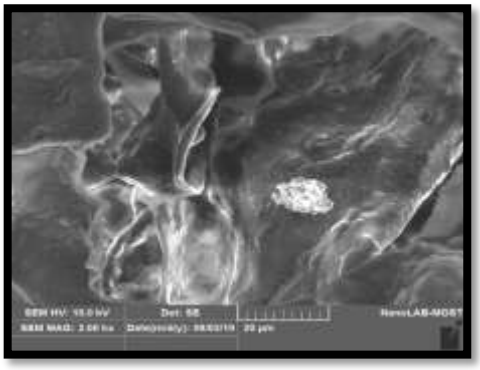


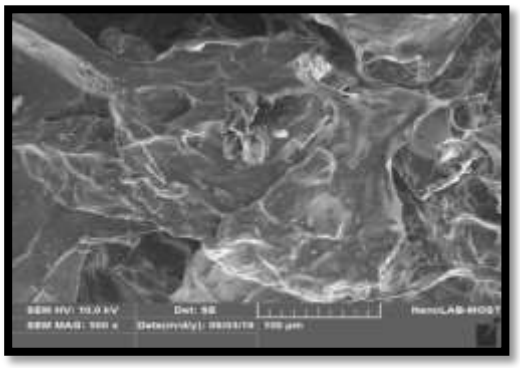
b) St. II





c) Bone ASC





d) Bone PSC

Figure (5): Morphological Comparative Study Pattern of Collagen Type (a).Naked eye ASC, (b) Naked eye PSC , (c)Standard I, (d) Standard II and (e) for ASC , (f) for PSC from the Bone of Catfish

Discussion

We found that aging-related increases in mineralization led to greater ash concentration in PSC bone catfish. However, due to biochemical makeup, the higher ash content found in the bone of the leather jacket could not be linked to the enhanced mineralization. Because the bone contains a fair amount of protein, it makes for suitable sources for collagen extraction. Fish species' muscles typically included between 54% and 80.3% of moisture and 16.1-27.9% of crude protein, respectively [15]. This species was referred to be garbage fish with minimal economic value because such a high concentration of protein was not observed in it. This discovery demonstrated the type I collagen status of ASC and PSC. Compared to carp bone collagen (2 is exactly 116 kDa), alligator (Alligator mississippiensis) bone collagen (1 and 2 chains are 123 and 110 kDa, respectively), and ASC and PSC subunits, which have molecular weights of roughly 110 kDa and 100 kDa, respectively, [17] Black drum and sheephead sea bream bone collagen (one and two chains are 130 and 110 kDa, respectively), [18] as well as the backbone collagen of Baltic cod (one and two chains are evident near 116 kDa). [19] High molecular weight components, such as and components, were also found in ASC and PSC. Higher percentages of components those with molecular weights of >200 kDa were present in HCl-PSC, and peptides those with molecular weights of 97 kDa were also present. These findings suggested that HCl-PSC underwent more chain breakdown than other collagens. Furthermore, HCl-PSC wasn't a type of collagen like collagen peptides or gelatin. Additionally, the SDS-PAGE pattern of the acid solution-residues utilized to remove bone minerals is shown Figure 2. Higher proportions, including the components and, were not discovered. On the other hand, many peptide fragments were identified. This outcome demonstrated that while de- saluting, a portion of the collagen in the bone was dissolved and broken down in HCl solution. The triple helix conformation, the spacing between molecular chains, and the distance between skeletons are all related to crystal and amorphous states. [20] Investigating collagen made from carp scales, he discovered that the wide peak's minimum d-spacing is (4.48) A o while the sharp peak's minimum d-spacing is (11.87) A o . X-ray diffraction is frequently used to evaluate collagen fibril distribution and orientation in fish, and it has been demonstrated from the X-ray diffractograms that the

morphological characteristics of the two types of collagen have a direct relationship with the phase structure of the material.tissues [21; 22]. In infrared spectroscopy, amide III is a weak beam that returns to the planar bending vibration of the NH group coupled with the stretching vibration of [27], The beam returns to the planar bending vibration of the NH group paired with the stretching vibration of the CN group due to amide bonding [28], in addition to the absorption coming from the oscillatory vibration of the CH2 group in the glycin and proline side chain structure. When pepsin cleaves the telopeptide region of tropo-collagen, the secondary structure of the resulting PSC may alter somewhat [29; 30]. The collagen from the skin of the magnificent squid had absorptions at 1031, 1060, and 1081 cm1, which are caused by the C-OH stretching vibrations of the carbohydrate moieties linked to the protein [31]. The finding revealed that collagens may be made up of carbohydrates that are joined to the polypeptide chain's hydroxylysine residues via O-glycosidic linkages. The outcome matched the amount of sugar in total as well. The outcome suggested that pepsin digestion during collagen extraction can have a minor impact on the triple helical structure. The coil-like fibrils were discovered as a sheet that was interconnected for ASC and as random windings of coil-like structures for PSC, respectively, which shows the collagen is fibrous. The microstructure of ASC and PSC differ significantly at high magnification, although the collagen of PSC appears to have a consistent, homogenous network with porous, honey-comb-like structure. This agrees with [32], who examined the exterior skin of the spineless cuttlefish Sepiella inermis and assessed the SEM for ASC and PSC.

.

References

1. Trevitt CR, Singh PN. Variant Creutzfeldt–Jakob disease: (2003). pathology, epidemiology and public ;78(3 Suppl):651S-656S. doi: 10.1093/ajcn/78.3.651S .
2. Pranoto Y, Lee CM, Park HJ. (2007) . Characterization of fish gelatin films added with gellan and κ-carrageenan processing of fish. Int J Food Sci Technol.;40(5):301–343.health implications. Am J Clin Nutr. 2003;78:651S–656S. [PubMed].
3. Sudeepa ES, Rashmi HN, Selvi AT, Bhaskar N. (2007). Proteolytic bacteria associated with fish processing waste: isolation and characterization. Int J. Food Sci Technol.;44(3):281–284.
4. Shahidi F. (1994).Seafood processing by-products. In: Shahidi F, Botta JR, editors. Seafoods chemistry, processing, technology and quality. Glasgow: Blackie Academic Professional; pp. 11–26.
5. Peck Loo Kiew and Mashitah Mat Don .(2013) . Modified Lowry's Method for Acid and Pepsin Soluble Collagen Measurement from Clarias Species Muscles. Volume 2 • Issue 3 p. (2-5). http://dx.doi.org/10.4172/scientificreports678.
6. Samantha, CFP. (2016) .Extraction of Collagen from Fish Wastes, Optimization and Characterization [master dissertation/thesis]. UTAR; http://eprints.utar.edu.my/2410/Google Scholar.
7. American Public Health Association. .1992. Compendium methods for microbiological examination of foods. 2nd ed., Washington, D. C.
8. Huang, Y., Shiau, C., Chen, H. and Huang, B. (2011). Isolation and Characterization of Acid and Pepsin-Solubilized Collagens from the Skin of Balloon Fish (Diodon holocanthus). Food Hydrocolloids, 25, 1507-1513. http://dx.doi.org/10.1016/j.foodhyd.2011.02.011.
9. Gornall, A. G., Bardawill, C. L. and David, M. M. (1949). Determination of Serum Protein by means of the biuret reaction. J.Biol. Chem. 177: 751-753.
10. Bilgin Vatansever, Merve Oztug Senal, Muslum Akgoz & Ahmet C. Goren. (2015). Development and validation of a generic method for quantification of collagen in food supplement tablets using liquid chromatography coupled with time-of-flight mass spectrometryAnalytical and Bioanalytical Chemistry volume 407, pages1981–1987.
11. Laemmli, U.K.(1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature.
12. Bama P., Vijayalakshimi M., Jayasimman R., Kalaichelvan P.T., Deccaraman M., Sankaranarayanan S..(2010).Extraction of Collagen Fromm Catfish(TACHYSURUS MACULATUS) by Pepsin Digestion and Preparation and Characterization of Collagen Chitosan Sheet. International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 2, Issue 4.
13. Michael Eckert.(2012).Max von Laue and the discovery of X-ray diffraction in 1912. Ann. Phys.(Berlin) 524, No. 5,A83-A85(2012) DOI 10.1002/andp.201200724.
14. Wang L.,X. An,Z. Xin,L. Zhao,Q. Hu.(2007). Isolation and Characterization of Collagen from the Skin of Deep-Sea Redfish (Sebastes mentella). Volume72, Issue8.Pages E450-E455. , 227, 680. [CrossRef].

15. Sato K, Yoshinaka R, Sato M, Shimizu Y. (1986 b) . Collagen content in the muscle of fishes in association with their swimming movement and meat texture. Bull Jpn Soc Sci Fisheries. ;52(9):1595–1600. doi: 10.2331/suisan.52.1595. [CrossRef] [Google Scholar].
16. Duan R.; J. Zhang ; X .Dua ; X. Yao ; K. Konno .2009. Properties of collagen from skin, scale and bone of carp (Cyprinus carpio) Food Chem.;112:702–706. doi: 10.1016/j.foodchem.2008.06.020. [CrossRef] [Google Scholar].
17. Wood, A.; Ogawa, M.; Portier, R.J.; Schexnayder, M.; Shirley, M.; Losso, J.N. (2008). Biochemical properties of alligator (Alligator mississippiensis) bone collagen. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 151, 246–249.
18. Sadowska, M.; Turk, M. (2010)Isolation and some properties of collagen from the backbone of Baltic cod ˙ (Gadus morhua). Food Hydrocolloids, 24, 325–329.
19. Ogawa, M.; Portier, R.J.; Moody, M.W.; Bell, J.; Schexnayder, M.A.; Losso, J.N.(2004).Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (Pogonia cromis) and sheepshead seabream (Archosargus probatocephalus). Food Chemistry, 88, 495–501.
20. Zhong S, Ma C, Lin YC, Luo Y. 2011. Antioxidant properties of peptide fractions rom silver carp (Hypophthalmichthys molitrix) processing by-product protein hydrolysates evaluated by electron spin resonance spectrometry. Food Chemistry. 126: 1636–1642.
21. Fengxiang, Z., Anning, W., Zhihua, L., Shengwen, H., Lijun, S. (2011).Preparation and characterisation of collagen from freshwater fish scales. Food Nutr Sci.;2(8):818-823.
Google Scholar.
22. Bigi, A.; Burghammer, M.; Falconi, R.; Koch, M.H.; Panzavolta, S.; Riekel, C. (2001).Twisted plywood pattern of collagen fibrils in teleost scales: An X-ray diffraction investigation. J. Struct. Biol., 136, 137–143.
23. Suda, H. K.; Kajiwara, M.; Matsumoto, N.; Murayama, H.; Yamato, H. (2009).Characterization of apatite and collagen in bone with FTIR imaging. International Journal of Food Properties, 505, 64–69. 17.
24. Kaminska, A.; Sionkowska, A.(1996) .Effect of UV radiation on the infrared spectra of collagen. Polymer Degradation and Stability, 51, 19–26.
25. Benedictode Campos Vidal ,Maria Luiza S.Mello.(2011).Collagen type I amide I band infrared spectroscopy.Micron ,Vol. 42, Issue 3, April 2011, Pages 283-289.
26. Yakimets, I.; Wellner, N.; Smith, A.C.; Wilson, R.H.; Farhat, I.; Mitchell, J. (2005).Mechanical properties with respect to water content of gelatin films in glassy state. Polymer, 46, 12577–12585.
27. Mansur, H.S.; Oréfice R.L.; Pereira, M.M.; Lobato, Z.I.P.;Vasconcelos, W.L. And Machado, L.J.C. (2002). FTIR And UV-Vis Study Of Chemically Engineered Biomaterial Surfaces For Protein Immobilization. Spectroscopy-An International Journal. 16 (3-4):351-360.
28. Plepis, A. M.; Goissis, G. D. and Das Gupta, D. K. (1996). Dielectric and pyroelectric characterization of anionic and native collagen. Polymer Engineering and Science., 36(24): 2932–2938.
29. Benjakul S., Thiansilakul Y., Visessanguan W. et al. (2010). Extraction and characterisation of pepsin-solubilised collagens from the skin of bigeye snapper (Priacanthus tayenus and Priacanthus macracanthus)," Journal of the Science of Food and Agriculture, vol. 90, no. 1, pp. 132–138,.View at: Publisher Site | Google Scholar.
30. Kittiphattanabawon P., Benjakul S., Visessanguan W., and Shahidi F. (2010). Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus)," LWT—Food Science and Technology, vol. 43, no. 5, pp. 792–800,.View at: Publisher Site | Google Scholar.
31. Petibois C., Gouspillou G., Wehbe K.,. Delage J.-P, and Déléris G. (2006). "Analysis of type I and IV collagens by FT-IR spectroscopy and imaging for a molecular investigation of skeletal muscle connective tissue," Analytical and Bioanalytical Chemistry, vol. 386, no. 7-8, pp. 1961–1966, 2006. View at: Publisher Site | Google Scholar.
32. Shanmugam Vairamani , Ramasamy Pasiyappazham, Subhapradha Namasivayam, Sudharsan Sadhasivan, Seedevi Palaniappan , Moovendhan Meivelu, Krishnamoorthy Jayalakshmi, Shanmugam Annaian and SrinivasanExtraction Alagiri.(2012). Extraction, structural and physical characterization of type I collagen from the outer skin of Sepiella inermis (Orbigny, 1848) . African Journal of Biotechnology Vol. 11(78), pp. 14326-14337.

الخصائص الوظيفية للكولاجين المستخلص من عظم سمك الجري

آيات عدنان عباس[1] خالدة عبد الرحمن شاكر[2]

[1]مركز بحوث التقنيات الاحيائية/ جامعة النهرين

[2]قسم علوم الاغذية / كلية الزراعة/ جامعة بغداد

الخلاصة :

الخلفية: يمثل الكولاجين 25 في المائة من جميع البروتينات الموجودة في الفقاريات. تشتق كلمة كولاجين من الكلمتين با اليونانية "kolla" و "genos" ، والتي تعني على التوالي "الغراء" و "التكوين". للكولاجين مجموعة واسعة من الاستخدامات البيولوجية والدوائية. توفر جلود الخنازير والبقر غالبية الكولاجين. تم تقييد استخدام الكولاجين الحيواني نتيجة لأوبئة أمراض الحيوانية معينة ، بما في ذلك مرض الحمى القلاعية (FMD) والتهاب الدماغ الإسفنجي البقري (FMD) (BSE)، والتي تشكل خطرًا لانتقال العدوى إلى البشر.

الهدف: الهدف من هذه الدراسة هو استخراج الكولاجين من عظم سمك السلور.

المواد وطرق العمل: تم استخدام عظام سمك الجري (Silurus triostegus) لاستخلاص الكولاجين الذي تم تحليله بطريقتين بالحامض والانزيم (ASC) .(PSC)تم قياس التركيب التركيبي للمستخلصات (الرطوبة ، البروتين ، الدهن ، الرماد) ، واستخدم الوزن الجاف لحساب ناتج ASC و .PSC تراوح الوزن الجزيئي للكولاجين اقيد الدراسة من 97 إلى 200 كيلو دالتون ، تبعا لطريقة الترحيل الكهربائي .SDS-PAGE من أجل تحديد كولاجين المجاميع الفعالة في العظام ، تم استخدام تحليل الأشعة تحت الحمراء (FTIR) لكلا من ASC و PSC المنتجة، بالإضافة إلى الكولاجين القياسي (النوع الأول والثاني). باستخدام المجهر الإلكتروني الماسح (SEM) ، تم الكشف عن التركيب المورفولوجي لـ ASC و (PSC) ، وتم تحديد زوايا الحيود (2 θ) لـ ASC و PSC باستخدام تحليل الأشعة السينية. ولتشخيص الكولاجين ، تم استخدام تقنيةHPLC .

النتائج: التركيب التقريبي لعظم ASC و PSC لسمك الجري. احتوت عظام سمك الجري على رطوبة (75.22٪) وكمية جيدة من البروتين (16.65٪) ودهون (2.40٪) ومحتوى رماد (0.28٪) ، بينما احتوت عظم PSC على رطوبة أقل قليلاً (68.50٪) من ASC ، تحتوي على نسبة عالية من البروتين (19.93٪) ، دهون (1.825٪) ، رماد(0.58) ٪, HPLC SEM، FTIR، الترحيل كهربائي ، الأشعة سينية.

الاستنتاج: عظام السمك كانت مصادر واعدة لتحضير الكولاجين ، الكولاجين القابل للذوبان في الحمض (ASC) ، الكولاجين القابل للذوبان في البيبسين (PSC) تم استخلاصه بنجاح بطريقتين. كان الكولاجين المستخرج من سمك السلور العظمي من النوع الأول من الكولاجين ، والذي يتكون من سلسلتين α1 وسلسلة α2 وسلسلة كما هو موضح في أنماط.SDS-PAGE

الكلمات المفتاحية: سمك الجري، العظام ، HPLC، الأشعة السينية ، FTIR، SDS، SEM، ASC، .PSC