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# Characterisation, Amino Acid Composition and Denaturation status of Acid Soluble Collagen from Catfish (*Clarias gariepinus*) Skin

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***Abstract:*** *This study reported characterisation of acid-soluble collagen from the skin of freshwater catfish (Clarias gariepinus) by Fourier Transform Infra-Red spectroscopy (FTIR), scanning electron microscopy (SEM) and energy dispersive x-ray (EDX). Amino acid composition and denaturation temperature of the collagen were determined. Acid soluble collagen (ASC) from the skin of C. gariepinus produced a comparatively yield of 2.38% with all amide functional groups (Amide A, B, I, II and III) visible in the FTIR spectrum suggesting the intactness of triple helical structure of the collagen. The SEM of C. glariepinus, shows a mono-fibrillated irregularly arranged crystalline surface material having 24.46 % carbon, 11.72 % oxygen and 9.40 % nitrogen. The abundance of amino acids follows glycine > arginine > proline > alanine indicating the integrity of collagen and a non-disruptive method of extraction. The denaturation temperature ($T_d$) of ASC was about 30 °C implying its usefulness in food and pharmaceutical industries.*

***Keywords:*** *Amide linkage, Clarias gariepinus, Denaturation, Gas chromatograph-pulse flame photometric detector*

## 1. Introduction

Collagen is the most abundant fibrous protein polymer in connecting tissues of animals especially fishes and it is a major component of the extracellular matrix (ECM) that accounts for approximately 30 % of total protein in the body (Mahboob, 2015; Gistelinck et al., 2016; Arumugam et al., 2018; Lim et al., 2019; Coppola et al., 2020; Jafari et al., 2020). It is composed of α-chain polypeptides arranged in triply unfolded helix surrounded by α-chains. It plays vital roles in the maintenance of functions of numerous tissues by providing flexibility alongside the provision of tensile strength for sustaining the integrity of the structure of biological macromolecules (Lim et al., 2019; Schmidt et al., 2016; Alves et al., 2017; Bhagwata et al., 2018). The wide applications of collagen due to its arrays of versatility, biodegradability, biocompatibility, availability and low antigenicity make it desirable in food, cosmetic, biomedical, film and leather industries. Owing to these properties, collagen has found usefulness in wound healing, coagulation of platelet, cell differentiation, as well as moisturizer, anti-ageing and anti-wrinkling agent in creams (Fujimaki et al., 2017; Hu et al., 2017; Liu et al., 2017; Subhan et al., 2017; Raman and Gopakumar, 2018; Jafari et al., 2020). It has equally become a vital ingredient in food processing as a food additive for improving rheological characteristics along with coating to extend shelf-life via prevention of oxidation and microbial growth (Chinh et al., 2019a; Coppola et al., 2020). Collagen has been extracted from fishes including channel catfish (*Ictalurus punctatus*) with properties suitable for applications in different industries. However, some of the extracted collagens suffer from possible contamination (Tan and Chang, 2018).

Nigeria is nearing food sustainability in fish production especially in catfish (*Clarias gariepinus*) production. Hence, the generation of bioactive biomaterial by-products is expected to be on the rise. Fish processing has surged and considerable waste is generated, thus, disposal of the huge fish waste majority such as bones, skin, scales, and fins that constitute over 70 % of fish could create environmental disturbance over time. Moreover, these discarded fish by-products are richly useful in collagen production (Adejumo et al., 2018; Abuine et al., 2019; Coppola et al., 2020).

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Extraction of collagen from fishes has received noteworthy attention as a substitute recently due to easier method of extraction, safety against zoonotic diseases, higher water solubility, enhanced biomaterial usage and better bioactive properties unlike from mammalian sources (Chen et al., 2016; Bhagwata et al., 2018; Lim et al., 2019; Tanaka et al., 2018; Kim et al., 2019). Some studies have reported limitations of low yield and lengthy extraction time in the extraction of collagen from catfish, tilapia and mackerel. Extraction and yield of collagen depend mostly on extraction procedure and type of fish. Although, collagen has been previously extracted from catfish (*I. punctatus*) (Tan and Chang, 2018), catfish of *C. gariepinus* specie has different compositions compared to *I. punctatus* and would not give similar yield as reported for the *C. gariepinus*. Therefore, this study aimed at improving percentage yield and reducing length of extraction procedure by using *C. gariepinus* for collagen production and characterizing the same for possible applications in industries. This study would also focus on the extraction of collagen from *C. gariepinus* to improve the commercial value of these cheaply abundant natural resources besides providing a new approach to finding alternative sources of safe collagen for industrial uses.

## 2. Materials and Methods

### 2.1 Collagen Extraction

Fresh farmed catfish (*C. gariepinus*) sample was bought at a local market in Osogbo, Osun State, Nigeria. It was stored in an ice pack and transported to the Laboratory of the Department of Pure and Applied Chemistry, Osun State University, Osogbo, Nigeria. The sample was washed with running tap water and de-skinned. The skin was washed with cold water (4-7 $^{o}$C), cut into small pieces and kept at -20 $^{o}$C.

### 2.2 Pre-treatment of Skin

Fish skin pre-treatment was carried out according to the method advocated by Singh et al. (2011). All processes were done at 4 $^{o}$C with continuous stirring. The prepared skin was mixed with 0.1 M NaOH at a skin/alkali solution ratio of 1:10 (w/v). The mixture was continuously stirred for 6 h and the alkali solution was changed every 2 h. The treated skin was then washed with cold water until a faintly basic pH (pH 7.8; Bio-base Precision pH/ORP meter, China) of wastewater was reached.

### 2.3 Acid Soluble Collagen (ASC) Extraction

Acid soluble collagen was extracted according to the method advocated by Nagai and Suzuki (2000) with modification. The pretreated skins were defatted with 10% butyl alcohol with a solid/solvent ratio of 1:10 (w/v) for 48 h with the 10 % butyl alcohol solution changed every 8 h. The defatted skin was washed with cold water 4-7 $^{o}$C followed by soaking in 0.5 M acetic acid with a solid/solvent ration 1:15 (w/v) for 24 h. The mixture was filtered through two layers of cheesecloth and the residue was re-extracted under the same conditions; both filtrates were combined. The collagen was precipitated by adding 2.6 M NaCl in the presence of 0.05 M tris (*hydroxylmethyl*) aminomethane (pH 7).

The resultant precipitate was collected by centrifuging at 20,000 g for 60 min using a refrigerated centrifuge (TGL 16 G, BBran Scientific and Instrument, England). The pellet was dissolved in 100 ml of 0.5 M acetic acid and dialyzed against 50 ml of 0.1 M acetic acid for 24 h followed by the dialysis in the same volume of distilled water for 24 h. The dialysate was freeze-dried and was referred to as acid-soluble collagen (ASC). The yield of ASC was calculated from the percentage of the dry weight of collagen extracted in comparison with the wet weight of the initial skin used (Equation 1).

$$\text{Yield (\%)} = \frac{\textit{weight of collagen obtained}}{\textit{weight of wet skin}} \text{ X } 100 \quad (1)$$

## 3. Characterization and Analysis

### 3.1 Fourier Transform Infra-red Spectroscopy

Acid soluble collagen was characterized by FTIR spectroscopy (Model Equinox 55, Bruker, Ettlingen, Germany) was done in the range of 400-4,000 $cm^{-1}$.

### 3.2 Morphology and Elemental Composition

Scanning electron microscopy coupled with energy dispersive X-ray was used to examine the microstructure of the collagen of *C. gariepinus*. The collagen sample was cut using a punch and fixed to an adhesive carbon stub. Imaging and elemental constituents were determined using SEM (Hitachi High-Technologies Corp., Japan).

### 3.3 Determination of Denaturation Temperature

The denaturation temperature was measured according to the method of (Nagai et al., 2001). 10 ml of 0.075 % collagen solution in 0.1 M acetic acid was used for the viscosity measurement using an Ostwald viscometer. The thermal determination curve was obtained by measuring solution viscosity at several temperatures from 10 °C to 50 $^{o}$C, the temperature was raised stepwise at each point. Fractional changes represent the change in viscosity with respect to temperature and was calculated from change in viscosity, as temperature was monitored (Chinh et al., 2019b).

### 3.4 Amino Acid Analysis

The amino acid content of the collagen was determined using Gas chromatography - pulse flame photometric detector (GC-PFPD). The method was modified according to that advocated by Oyedeji et al. (2017) and Adejumo et al. (2018). The dried and pulverized sample was made to be free of water by ensuring constant weight for a while in the laboratory. Ten grams (10g) of

the sample was defatted with 30 ml petroleum spirit three times and thereafter hydrolysed three times for complete hydrolysis to be achieved for the total amino acid determination. Both the pulverized sample and defatted sample were soaked with 30 ml of the 1.0 M potassium hydroxide solution and incubated for 48 h at 110 ℃ in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralised to get the range of 2.5-5.0. The purified solutions were derivatized with ethyl choroformate.

The derivatizing agent was afterwards removed by passing under streams of nitrogen. Aliquots of amino acids derivatives dissolved in dichloromethane were analysed by gas chromatography equipped with pulse flame photometric detector (GC-PTFD - HP 6890). 1 µl of concentrate was injected into GC-PFPD using (30 m × 0.25 mm × 0.255 mm ID) HP5 column for individual amino acid peaks. The initial temperature of the hydrogen carrier gas and column was 60 ℃. It was ramped at 8 ℃ for 20 min and held constant for 2 min and then at 12 ℃/min for 6 min and held for 2 min.

Amino acids standard solutions were repeatedly analysed five (5) times and calibration curves obtained had correlation coefficients between 0.9996 and 0.9999.

## 5. Results and Discussion

### 5. Percentage Yield of Collagen from *C. gariepinus* Skin

Acid soluble collagen extracted with acetic acid from the skin of catfish yielded 2.38 %. The yield of collagen obtained in the study from *C. gariepinus* is comparably higher than the percentage collagen yields reported for skins of sole fish (1.93 %) and Brama australis (1.5 %) (Sionkowska et al., 2015). Generally, collagen yields are affected by temperature, extraction time, concentration of acetic acid, collagen structure and other variables (Alves et al., 2017; Bhagwata et al., 2018; Tan and Chang, 2018).

### 5.2 FTIR Characterisation

Acid soluble collagen from *C. gariepinus* was characterised by Fourier Transform Infra-Red spectroscopy and showed representative peaks indicating the presence of amide linkage (see Figure 1). The peak at 3,285 $cm^{-1}$ represents N-H stretching amide A, while peak at 1,632 $cm^{-1}$ is attributable to C=O stretching vibration characteristic of amide I in proteins. Peaks at 2,923 (amide B) and 2,879 $cm^{-1}$ are representative of C-H stretching vibrations of $CH_3$ and $CH_2$, respectively. Peaks at 1,542 $cm^{-1}$ imply amide II indicating the presence of NH vibration associated with CO stretching while 1,240 $cm^{-1}$ represents amide III corresponding to C-H stretching that gives information about the helical structure of collagen. The presence of amide A, B, I, II and III imply no disorganisation in the triple helical structure of collagen (Coppola et al., 2020; Pal and Suresh, 2016; Wang et al., 2018).

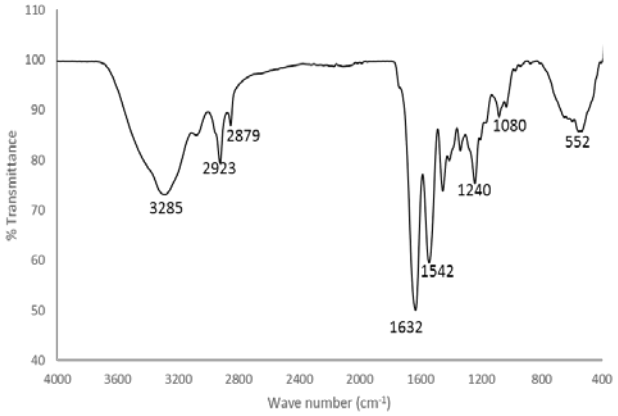


**Figure 1.** FTIR Spectrum of Acid Soluble Collagen from C. gariepinus

The result of spectra characterisation of collagen from *C. gariepinus* in this study conforms with results of collagen extracted from the bladder of *Gadus Morhua*, scales of freshwater carp fish, sole fish skin and marine fish skin (Arumugam et al., 2018; Alves et al., 2017; Chinh et al., 2019b). The FTIR spectrum information obtained for collagen in this study is comparable with gelatin previously reported (Adejumo et al., 2018).

### 5.3 Morphology and Elementation Composition of *C. gariepinus* Collagen

Scanning electron micrograph of collagen from *C. gariepinus* shows a mono-fibrillated irregularly arranged crystalline surface material (see Figure 2) having prominently carbon (24.46 %), followed by oxygen (11.72 %), nitrogen (9.40 %), chlorine (3.26 %), sodium (0.64 %) and aluminium (0.24 %) (see Figure 3). The range of prominent elements in collagen usually follows C > O > N with Cl and Na coming from the extraction procedure (Chinh et al., 2019a, 2019b). These elements represent the constituents of polypeptide linkage in collagen.

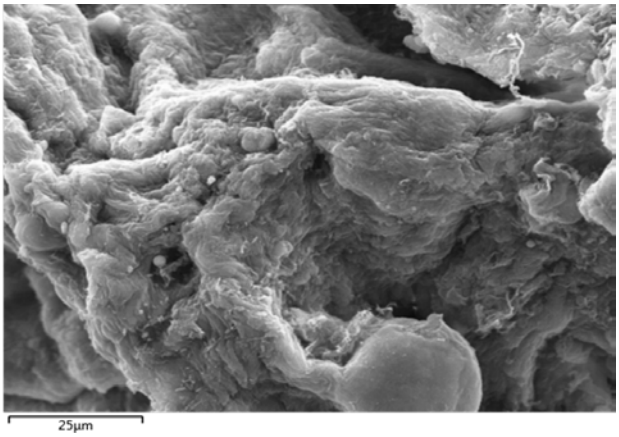


**Figure 2a.** Scanning electron micrograph of collagen extracted from *C. gariepinus*

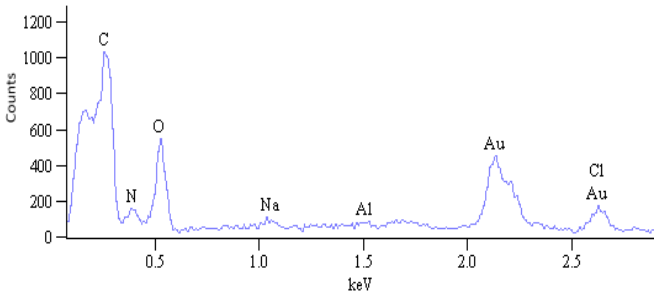


**Figure 3.** EDX Spectrum Showing Elemental Composition of Collagen from *C. gariepinus*

### 5.4 Amino Acid Composition of *C. gariepinus* Collagen

Acid soluble collagen from *C. gariepinus* (see Table 1) analysed with GC-FID contained seventeen (17) amino acids (nine essential and eight non-essential) with glycine. The most abundant amino acid followed by arginine, proline and alanine. The prominence of glycine alongside a high quantity of other amino acids especially proline in the *C. gariepinus* collagen is similar to reports of collagen by Chinh et al. (2019b), Wang et al. (2018) and Tylingo et al. (2016). This aligns with the report that collagen is composed of a continuously repeating sequence of glycine-proline-hydroproline (Garehgheshlagh et al., 2020; Pal and Suresh, 2016; Coppola et al., 2020).

**Table 1.** Amino Acid Composition of C. gariepinus Collagen

| Composition (g/100 g protein) | | | |
|---|---|---|---|
| Essential Amino Acids | | Non-Essential Amino Acids | |
| Isoleucine | 1.13 | Glycine | 25.45 |
| Leucine | 1.60 | Alanine | 8.27 |
| Lysine | 1.09 | Serine | 2.21 |
| Methionine | 0.32 | Proline | 9.35 |
| Cystine | 0.04 | Aspartate | 2.16 |
| Phenylalanine | 0.75 | Glutamate | 2.40 |
| Tyrosine | 0.04 | Histidine | 0.08 |
| Threonine | 1.16 | Arginine | 12.02 |
| Valine | 1.38 | | |

Similarly, glycine is usually the most abundant amino acid in collagen due to its presence in fishbone, scales, and fins while proline is needed for maintaining the integrity of collagen structure and for thermal stability (Arumugam et al., 2018; Lim et al., 2019; Bhagwata et al., 2018; Wang et al., 2018). In comparison with amino acid composition of gelatin extracted from comparable catfish, collagen contained different amino acid components, though, both are proteinous but differences exit (Adejumo et al., 2018).

### 5.5 Denaturation Temperature of Collagen from *C. gariepinus* Skin

The denaturation temperature indicates the heat-induced changes in the structure of collagen when the viscosity of collagen solution is 0.5 (Chinh et al., 2019b). As obtained in this study, the denaturation curve displayed an endothermic peak corresponding to denaturation temperature ($T_d$) of 30 $^oC$ (see Figure 4).

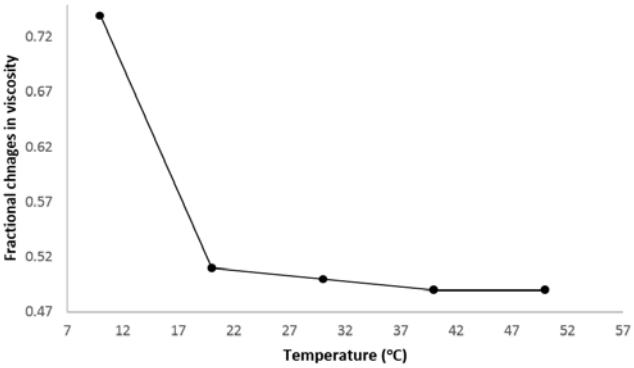


**Figure 4.** Thermal Denaturation Plot of Extracted Collagen from C. gariepinus

This value is similar to *C. gariepinus* collagen (29.3 $^oC$) by Tylingo et al. (2016) and is consistent with the range of results of $T_d$ for collagen from scales of freshwater carp fish (32.2 $^oC$), big-eye snapper fish (30.4 $^oC$), brown striped snapper (31.5 $^oC$) (Chinh et al., 2019b). The slight difference in the values of this study and other reported studies could be due to difference in the fish environment, amino acid composition and physiological temperature of fish. The $T_d$ of Collagen extracted in this study implies that it would find a useful application in the pharmaceutical and food industries (Tylingo et al., 2016; Garehgheshlagh et al., 2020; Jafari et al., 2020).

## 6. Conclusion

Acid soluble collagen was successfully isolated from the skin of catfish and characterised without structural disruptions and deformations. A relatively high yield was obtained and it contained all amide linkages responsible for collagen structure. A relatively moderate denaturation temperature of 4 °C is an indication of its usefulness as a food additive and for other pharmaceutical purposes. A good range of amino acids with a prominence of glycine and proline are indicators of the integrity of acid-soluble extracted in this study.

## References:

Adejumo, A.L., Aderibigbe, F.A. and Owolabi, R.U. (2018), "Chemical composition and characterisation of skin gelatins from two different freshwater fish species in Osun State of Nigeria: A comparative study", *The West Indian Journal of Engineering*, Vol.41, pp.47-54.

Alves, A.L., Marques, A.L.P., Martins, E., Silva, T.H. and Reis, R.L. (2017), "Cosmetic potential of marine fish skin collagen", *Cosmetics*, Vol.4, pp.39-54

Arumugam, G.K.S., Sharma, D.B.R.M., Ettiyappan, J. and Babu, P. (2018), "Extraction, optimisation and characterisation of

collagen from sole fish skin", *Sustainable Chemistry and Pharmacy*, Vol.9, pp.19-26.

Bhagwata, P.K. and Dandge, P.B. (2018), "Collagen and collagenolytic proteases: A review", *Biocatalysis and Agricultural Biotechnology*, Vol.15, pp.43-55.

Chen, S., Chen, H., Xie, Q., Hong, B., Chen, J., Hua, F. and Wu H, (2016), "Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenops ocellatus*)", *Food Hydrocolloids*, Vol.52, pp.468-477.

Chinh, N.T., Manh, V.Q., Trung, V.Q., Trang, T.D.M. and Hoang, T. (2019a), "Extraction of hydroxyapatite and collagen from the Vietnamese tilapia scales", *Vietnam Journal of Chemistry*, Vol.57, pp.225-228

Chinh, N.T., Manh, V.Q., Trung, V.Q., Lam, T.D., Huynh, M.D., Tung, N.Q., Trinh, N.D., Hoang, T. (2019b), "Characterisation of collagen derived from tropical freshwater carp fish scale wastes and its amino acid sequence", *Natural Product Communications*, Vol.12, pp. 1-12.

Coppola, D., Oliviero, M., Vitale, G.A., Lauritano, C., D'Ambra, I., Iannace, S. and Pascale D. (2020), "Marine collagen from alternative and sustainable sources: Extraction, processing and applications", *Marine Drugs*, Vol.18, pp.214.

Fujimaki, H., Uchida, K., Inoue, G., Miyagi, M., Nemoto, N., Saku, T., Isobe, Y., Inage, K., Matsushita, O., Yagishita, S., Sato, J., Takano, S., Sakuma, Y., Ohtori, S., Takahashi, K. and Sato, J. (2017), "Oriented collagen tubes combined with basic fibroblast growth factor promote peripheral nerve regeneration in a 15 mm sciatic nerve defect rat model", *Journal of Biomedical Material Research*, Vol.105, pp.8-14

Garehgheshlagh, N.S., Fatemi, M.J., Jamili, S.H., Sharifi, A.M. and Nourani, M.R. (2020), "Isolation and characterisation of acid-soluble collagen from the skin of Rutilus Frisii Kutum (*Kamensky*) of the Caspian Sea", *Iranian Journal of Fisheries Sciences*, Vol.19, pp.768-779.

Gistelinck, C.G., Gagliardi, A., Tonelli, F., Marchese, L., Bianchi, L. and Staes, A. (2016), "Zebrafish collagen type I: molecular and biochemical characterisation of the major structural protein in bone and skin", *Scientific Reports*, Vol. 6, pp.21540-21554.

Hu, Y., Dan, W., Xiong, S., Kang, Y., Dhinakar, A., Wu, J. and Gu, Z. (2017), "Development of collagen/polydopamine complexed matrix as mechanically enhanced and highly biocompatible semi-natural tissue engineering scaffold", *Acta Biomaterial*, Vol.47, pp.135-148.

Jafari, H., Lista, A., Siekapen, M.M., Bohlouli, P.G., Nie, L., Alimoradi, H., and Shavandi, A. (2020), "Fish collagen: Extraction, characterisation, and applications for biomaterials engineering", *Polymers*, Vol.12 (10), pp.2230-2266.

Kim, H., Jang, J., Park, J., Lee, K.P., Lee, S., Lee, D.M., Cho and D.W. (2019), "Shear-induced alignment of collagen fibrils using 3D cell printing for corneal stroma tissue engineering", *Biofabrication*, Vol.11, pp.35-17.

Lim, Y., Ok, Y., Hwang, S., Kwak, J. and Yoon, S. (2019), "Marine collagen as a promising biomaterial for biomedical applications", *Marine Drugs*, Vol.17, pp. 467-499

Liu, C., Liu, X., Xin, Y., Cheng, X., Xu, T., Cha, D. and Fan, B. (2017), "Fabrication and Evaluation of thermo sensitive chitosan / collagen / α. Carbohydrate", *Polymer*, Vol.167, pp.145-157.

Mahboob, S. (2015), "Isolation and characterisation of collagen from fish waste material-skin, scales and fins of Catlacatla and Cirrhinusmrigala", *Journal of Food Science and Technology*, Vol.52, pp.4296-4305.

Nagai, T. and Suzuki, N. (2000), "Isolation of collagen from fish waste material-skin, bone and fins", *Food Chemistry*, Vol.68, pp.277-281.

Nagai, T., Yamashita, E., Taniguchi, K.K. and Suzuki, N. (2001), "Isolation and characterisation of collagen from the outer skin waste material of cuttlefish (*Sepia lycidas*)", *Food Chemistry*, Vol.72, pp.425-429.

Oyedeji, O.A., Azeez, L. and Osifade, B.G. (2017), "Chemical and nutritional compositions of flame of forest (*Delonix regia*) seeds and seed oil", *South African Journal of Chemistry*, Vol.70, pp. 16-20.

Pal, G.K. and Suresh, P.V. (2016), "Sustainable valorisation of seafood by-products: Recovery of collagen and development of collagen-based novel functional food ingredients", *Innovative Food Science and Emerging Technologies*, Vol.5, pp.13-19.

Raman, M. and Gopakumar, K. (2018), "Fish collagen and its applications in food and pharmaceutical industry: A review", *EC Nutrition*, Vol.13, pp.752-767.

Schmidt, M.M., Dornelles, R.C.P., Mello, R.O., Kubota, E.H., Mazutti, M.A., Kempka, A.P. and Demiate, I.M. (2016), "Collagen extraction process", *International Food Research Journal*, Vol.23, pp.913-922.

Singh, P., Benjakul, S., Maqsood, S. and Kishimura, H. (2011), "Isolation and characterisation of collagen extracted from the skin of striped catfish (Pangasianodon hypophthalmus)", *Food Chemistry*, Vol.124, pp.97-105.

Sionkowska, A., Kozłowska, J., Skorupska, M. and Michalska, M. (2015), "Isolation and characterisation of collagen from the skin of Brama australis", *International Journal of Biological Macromolecule*, Vol.80, pp.605-609.

Subhan, F., Kang, H.Y., Lim, Y., Ikram, M., Baek, S.Y., Jin, S., Jeong, Y.H., Kwak, J.Y. and Yoon, S. (2017), "Fish scale collagen peptides protect against CoCl2/TNF- α-induced cytotoxicity and inflammation via inhibition of ROS, MAPK, and NF-κ B pathways in HaCaT cells", *Oxidative Medicine and Cellular Longevity*, pp. 1 - 8. https://doi.org/10.1155/2017/9703609

Tan, Y., Chang, S.K.C. (2018), "Isolation and characterisation of collagen extracted from channel catfish (*Ictalurus punctatus*) skin", *Food Chemistry*, Vol.242, pp.147-155

Tanaka, T., Takahashi, K., Tsubaki, K., Hirata, M., Yamamoto, K., Biswas, A. and Kawamura Y. (2018), "Isolation and characterisation of acid-soluble bluefin tuna (*Thunnusorientalis*) skin collagen", *Fisheries and Aquatic Sciences*, Vol.21, pp.7-15.

Tylingo, R., Mania, S., Panek, A., Piątek, R. and Pawłowicz, R. (2016), "Isolation and characterisation of acid soluble collagen from the skin of African catfish (*Claria sgariepinus*), salmon (*Salmo salar*) and Baltic cod (*Gadus morhua*)", *Journal of Biotechnology and Biomaterial*, Vol.6, pp.234-240

Wang, J., Pei, X., Liu, H. and Zhou, D. (2018), "Extraction and characterisation of acid-soluble and pepsin-soluble collagen from skin of loach (*Misgurnus anguillicaudatus*)", *International Journal of Biological Macromolecules*, Vol.106, pp.544-550.

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