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PARTIAL CHARACTERIZATION OF COLLAGEN SHEET FROM LUTJANUS CAMPECHANUS SCALE

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ABSTRACT

The purpose of this study is to prepare fish scale collagen sheet from red snapper (Lutjanus campechanus) scales and to study its characterization. Fish scale waste became by-product from the fish processing industry, and utilization of these by-products to produce valuable collagen can give benefit to the entire world. From this study, collagen sheets were successfully prepared from fish Lutjanus campechanus scale waste. The fish scales obtained from local markets in Terengganu were washed thoroughly and dried. The scales were then being treated with different concentration of HCl, pulverized and paste into sheets. The effect of concentration of HCl on the fish scales, the effect of thickness to collagen sheet tensile strength and the component inside collagen sheet were determined. The effect of concentration of HCl shows that, 2.8 M is the best concentration to prepare fish scale collagen sheet. The tensile strength has no correlation with the thickness of collagen sheet, and the tensile strength which is in average of 29 MPa is enough to use the sheet as a wound dressing material. The components inside the collagen sheet show the same component as the previous study, which is the high characteristic of collagen. From the Thin Layer Chromatography (TLC) TLC analysis shows that fish scale collagen contains the abundance of Leucine amino acid. From this study, Lutjanus campechanus fish scale waste was successfully made into sheets. T and 3he components inside the fish scale collagen sheet also can be normally observed in bovine and porcine collagen, which conclude that fish collagen can be an alternative to mammalian collagen.

Keywords: fish scale, by-product, wound dressing, amino acid.

INTRODUCTION

Collagen is a protein made up of amino-acids, which are in turn built of carbon, oxygen and hydrogen. Collagen contains specific amino acids which are Glycine, Proline, Hydroxyproline and Arginin [6]. Collagen makes up approximately 30% of the proteins within the body.

Collagen contributes about 25% of vertebrate total proteins [9]. Generally, collagen has been extracted from the skins of some vertebrate species like cattle and pig. However, collagen from fish source is less common. Previous studies on fish collagen involved fish such as from paper nautilus [7]; big eye snapper [2] and Threadfin bream [8]. The parts of fish that can be used as a source of collagen are bone, fin, and skin. According to [9], the bones and scales of fish contain a huge amount of collagen like other vertebrates.

This study will utilize the abandoned fish waste produced by fish processing industry, since the scales of fish can be a useful source of collagen. Fish scales may have the potential to be used as an alternative to mammalian collagen. There are many reasons to substitute cattle and pig collagen with fish collagen. For example, the existence of mad cow disease makes the consumer feel unsafe to consume collagen from cattle. Besides that, the uses of pig collagen have been prohibited due to religious constraints. Therefore, a demand for alternative safe and permissible source of collagen is increasing [9]. Collagen also has the ability to promote cell attachment and proliferation, and subsequently can be used as a wound dressing material [1].

There are 50-70% of the original raw materials generated as a waste in fish shops and processing factories, and fish scale constitutes a major solid waste [5]. Improper disposal techniques cause environmental pollution resulting in offensive odor., Ttherefore, a proper optimized utilization of these wastes into valuable end products will be a promising solution.

Furthermore, the extraction process for Piscean collagen preserves its triple-helix structure. Dressings containing Piscean collagen have demonstrated a high level of biocompatibility., Wwhile animal studies have found that collagen extracted from fish did not produce an immunological response or allergic reactions, and was comparable to the type I bovine collagens [10].

Hence, the purpose of this study is to prepare a fish scale collagen made from red snapper (Lutjanus campechanus) scales, to determine the effect of different concentration of hydrochloric acid on fish scales during treatment, to study the effect of the thickness of the fish scale collagen sheet to the tensile strength of the prepared fish scale collagen sheet for suitability for wound dressing material, and to study the component in fish scale collagen sheet by using Fourier Transform Infrared (FTIR) FTIR and Thin Layer Chromatography (TLC)TLC.

MATERIALS AND METHOD

Fish scales from the fish of Lutjanus Campechanus were used as the source of fish collagen. The scales were collected at the local market and were washed thoroughly in running water and dried under the sun for 5 hours. The dried scales were stored in the dry polyethylene bag at room temperature until used.

Preparation of fish scales collagen sheet

The hydrochloric acid was diluted from 12 M to 2.2 M, 2.8 M and 3.4 M. The scales were treated with the diluted hydrochloric acid at room temperature for 24 hours in 3 separate beakers with a ratio of 1: 2, w/v, fish scales/HCl, as 100 g fish scales/200 ml HCl. After 24 hours, the acid was decanted and the scales are washed with distilled water. The scales were then being pulverized with distilled water with a ratio of 1:1 that 100 g fish scales/100 ml water for 15 minutes by using a domestic mixer. The resulting paste was cast into sheet and dried at room temperature.

Fourier transform infrared (FTIR) spectroscopy

The prepared fish scale collagen sheet was cut into a dimension of 20 mm x 20 mm and tested by using FTIR machine.

Tensile strength

Four samples of fish scale collagen sheet with 4 different thicknesses of 1.0 mm, 1.5 mm, 2.0 mm and 2.4 mm, were tested by using universal tensile machine. The fish scale collagen sheet was cut into dimension of 110 mm x 40 mm to gauge length of 70 mm for each sample. The sample was tested for its tensile strength and the value was observed.

Thin layer chromatography (TLC)

The eluent was prepared by mixing n-butanol, acetic acid and distilled water in a ratio of 5:1:5. The solution was stirred for 10 minutes, then let the layers separate. The starting line to the paper was marked 8 mm from the edge of the plate with graphite pencil. The locations where the samples will be spotted were also marked. The spots of collagen which have been prepared earlier and sample standard solutions which is fish oil are applied to the chromatographic paper by using a dropper. After application of samples, the spot was left to dry. Meanwhile, measure with a graduated test-tube 5 ml of eluent into the elution chamber. The chamber was covered with lids to let the chamber atmosphere saturate with eluent vapors for around 10 minutes. Then, the chromatographic paper was inserted into the elution chamber. The level of eluent must be on the line that has been marked earlier. After around 20 minutes, the paper was then removed from elution chamber and left to dry. After 2-3 minutes mark the eluent front with pencil and dry the paper in the oven for 5 minutes. When the paper is dry, the paper was viewed under the ultraviolet (UV) light to see the eluent movement. The eluent was marked and the Rf was calculated.

RESULTS AND DISCUSSIONS

The collagen sheet was successfully developed and has a prospect of being used as a wound dressing material. The source of collagen was the demineralized scales of Lutjanus Campechanus. Pure collagen was not isolated in this study. The moderately demineralized scales, thus obtained could easily be pulverized into a smooth paste. The prepared fish scale collagen sheet is as in Figure-1.

Figure-1. Collagen sheet from fish scales.

FTIR spectra of the fish scale collagen sheet are shown in Figure-2. The region of amide I, amide II and amide III are known to be directly related to the configuration of a polypeptide. Amide I band (1630.7 cm-1) is associated with stretching vibrations of carbonyl groups (C = O bond) in peptides, which being the most important factor in investigating the secondary structure of a protein. Amide II at 1541.06 cm-1 is associated with NH bending and CN stretching. Amide III (1235.68 cm-1) is related to CN stretching and NH, and involved with the triple helical structure of collagen. Peaks at 1060.75 cm-1 and 1135.85 cm-1 represents P-H bending and phosphate stretching respectively, which indicates the presence of calcium salts. The peak at 2927.97 cm-1 represents the CH2-CH3 vibrations, which are the characteristic of collagen. Peaks at 866.99 cm-1 and 938.85 cm-1 represent the presence of hydroxyproline and proline respectively which is the component for triple helical structure of collagen (Gly-Pro-X).

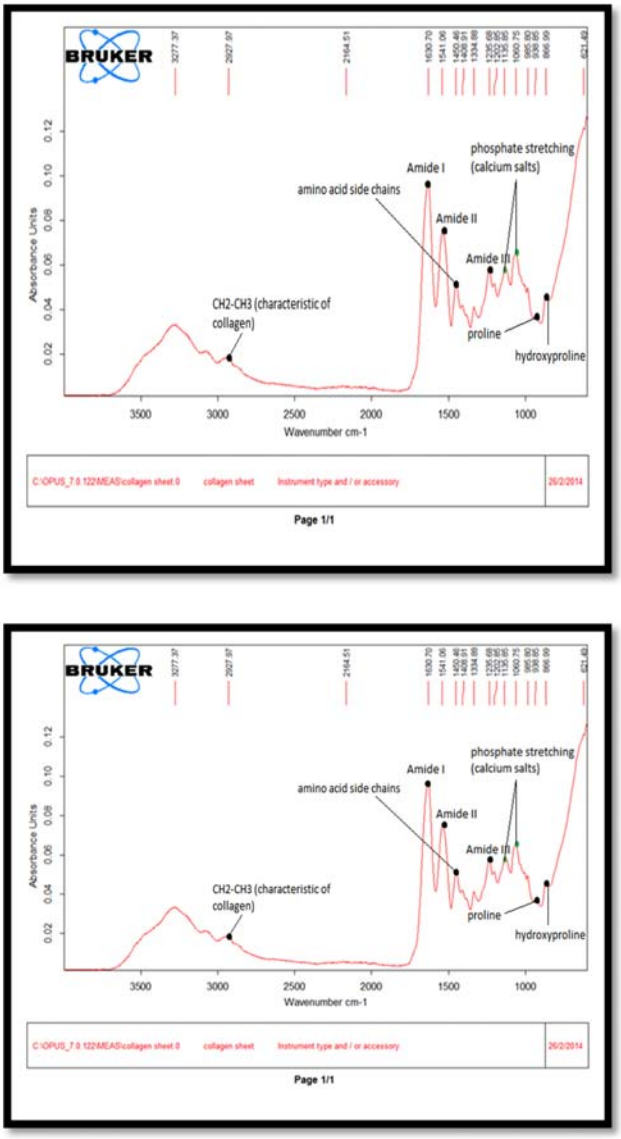

Figure-2. FTIR spectra of fish scale collagen sheet.

Table-1. FTIR spectra peak location for fish scale collagen sheet.

Peak positions (cm^{-1})	**Assignments**	
866.99	$_{v}$(CC), $_{\delta}$(CCH)	Hydroxyproline
938.85	$_{v}$(CC), $_{\delta}$(CCH)	Proline
1060.75	$_{\delta}$(PH)	Calcium salts
1135.85	$_{v}$(PH)	Calcium salts
1235.68	$_{v}$(CN), $_{\delta}$(NH)	Amide III
1450.46	$_{v}$(CH_2, CH_3)	Amino acid side chains
1541.06	$_{\delta}$(N-H)	Amide II
1630.70	$_{v}$(C = O)	Amide I
2927.97	$_{v}$(CH_2)	Amide B
3277.37	$_{v}$(NH), $_{\delta}$(OH)	Amide A

Legend: v-stretching mode, δ-bending mode

Based from FTIR spectra, we can see that the presence of different protein component that represent the characteristics of collagen. The P-H bending and phosphate stretching bands indicate that there is an inorganic phase in the fish scale collagen sheet. This obviously shows that fish scales were partially demineralized. The components inside the fish scale collagen sheet also can be normally observed in bovine and porcine collagen, which concludes that fish collagen can be an alternative to mammalian collagen [11].

Effect of thickness of fish scale collagen sheet to tensile strength

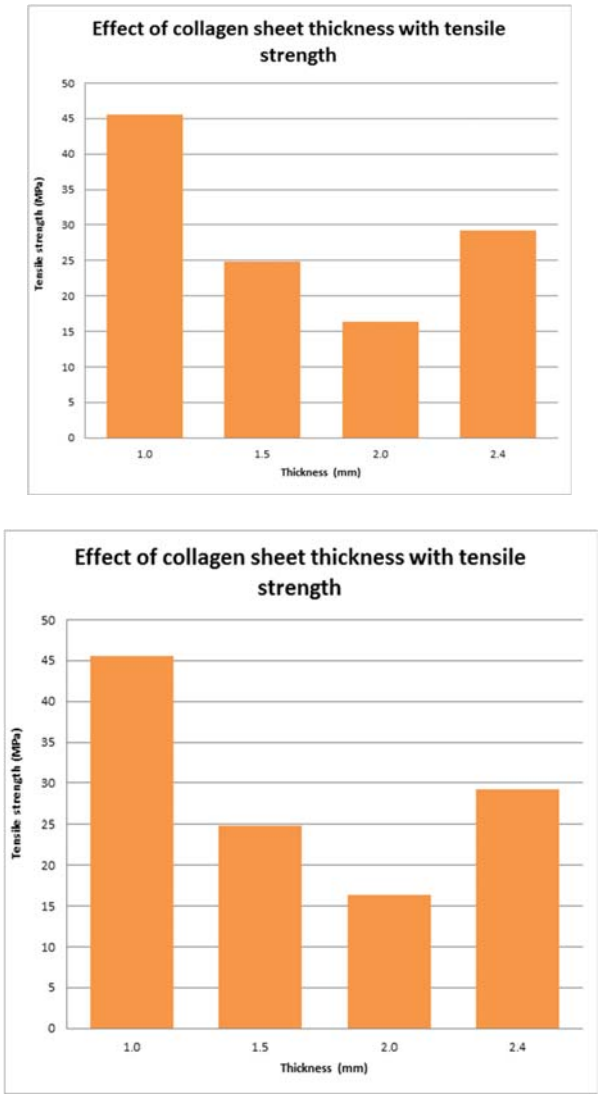

Figure-3. Effect of collagen sheet thickness with tensile strength.

According to figure-3, the ultimate tensile strength measured for the 1.0 mm, 1.5 mm, 2.0 mm and 2.4 mm samples were 45.604 MPa, 24.859 MPa, 16.484 MPa, and 29.238 MPa respectively. It was observed that the intensity of the lateral arrangement of the collagen molecules inside the fibrils, increased linearly with the strain. This was interpreted as a reduction of the disorder in the lateral molecular packing within fibrils, which resulting from the straightening of component in the collagen. From the result in Figure- 3, it can be seen that the thickness of fish scale collagen sheet had no significant influence on the overall value recorded.

Raw fish scales have a very high tensile strength which is around 90 MPa because of due to the hierarchically ordered structure of mineralized collagen fibres. In contrast, demineralized scales have significantly lower tensile strength which is in average around 29 MPa. This indicates that interactions between the apatite crystals and collagen fibres are of fundamental importance in determining the mechanical properties. The average tensile strength was very low as the demineralized scales were ground into paste and reconstituted into a sheet. and nNaturally, the reconstituted sheets will have poor tensile strength. However, the tensile strength of the collagen sheet prepared is sufficient to handle when it is used as a wound dressing material.

The fish scale collagen sheet is proposed to be used on wound dressing material on leprosy, diabetic and other chronic ulcers. As the use of bovine collagen may be restricted in future due to mad cow and foot and mouth diseases, the collagen from other sources have to be tried as wound dressing materials and for other pharmaceutical uses. In this direction, the collagen sheet may be an alternative to the bovine collagen. The porous nature of sheet will help to absorb the wound fluid when it is applied on wound, and thereby keeping wound dry. and tThis property helps in enhancing the rate of healing of the wound.

Thin layer chromatography (TLC)

After the complete run in the chromatographic chamber, TLC plate was air dried and the spot was observed under the UVlight. Rf values were subsequently determined for the separated amino acids. The results are calculated as below:

Rf = Distance travelled by substance

Distance travelled by solvent front (1)

According to Meditsiiniline, 2012[11], the calculated Rf exhibit the present of amino acid, that to be specific abundant of leucine. The standard of fish oil is used for comparison with sample collagen. The Rf values obtained from the investigation which is 0.84 are nearly similar to the standard values which is 0.88.

Based on previous studies, Glycine was the most prevalent amino acid in collagens [11] followed by proline and hydroxyproline as the amounts of proline and hydroxyproline are important for the structural integrity of collagen. However, based on the result, the abundance amino acid found was leucine. This may because some components may have such similar polarities that they appear under one spot after development. It might be the polarity of the development solvent is too high that all components in the mixture moves along with the solvent thus prevailing only leucine.

CONCLUSIONS

Based on the experiment which carried out, it can be conclude that the fish scale collagen has a great potential as an alternative to bovine and pig collagen. The collagen sheet is successfully prepared, and it can be used as a wound dressing material based on the characteristic exhibited. From the FTIR result, it shows the component of collagen that include various type of amino acid like proline and hydroxyproline which are the component of triple helix collagen. The tensile strength were also enough to be used as wound dressing material. The thickness of the collagen sheet has no correlation with the tensile strength of collagen sheet. Which it shows the structured and fibrils inside the sheet that affect the tensile strength of the collagen sheet. The concentration of hydrochloric acid affects the fish scales and the optimum concentration to prepare fish scale collagen sheet is 2.8 M. The TLC analysis exhibit the abundance of leucine in collagen sheet. The collagen sheet from fish Llutjanus campechanus scales can be successfully prepared. and tThe collagen is thought to be a viable replacement for bovine and porcine collagens with the advantages of being disease free, without the risk for bovine spongiform encephalopathy (BSE) BSE and foot/mouth disease.

REFERENCES

[1] S. K. Basha, R. V. S. Kumar, V. Haragopal, C. Srilatha, T. P. Sastry and M. Vidyavathi. 2011. Effects of fish scales extracted collagen biocastings oncutaneous wound healing in dogs. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2(2): 36-49.

[2] P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, T. Nagai and M. Tanaka. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeyesnapper (Priacanthus tayenus). Food Chemistry. 89(3): 363-372.

[3] Lodish H. 2008. Molecular cell biology. Macmillan.

[4] C. Meena, S. A. Mengi and S. G. Deshpande. 1999. Biomedical and industrial applications of collagen. Proceedings of the Indian Academy of Sciences-Chemical Sciences. 111(2): 319-329.

[5] T. Muthukumar, R. Senthil and T. P. Sastry. 2013. Synthesis and characterization of biosheet impregnated with Macrotyloma uniflorum extract for burn/wound dressings. Colloids and surfaces B: Biointerfaces. 102 (1): 694-699.

[6] Mandal A. 2014. Collagen-What is collagen? News Medical. http://www.news-medical.net/health/What-is-Collagen.aspx.

[7] T. Nagai, Y. Araki and N. Suzuki. 2002. Collagen of the skin of ocellate puffer fish (Takifugu rubripes). Food Chemistry. 78(2): 173-177.

[8] S. Nalinanon, S. Benjakul, W. Visessanguan and H. Kishimura. 2008. Tuna pepsin: Characteristics and its use for collagen extraction from the skin of threadfin bream (Nemipterus spp.). Journal of Food Science. 73(5): C413-C419.

[9] M. Ogawa, M. W. Moody, R. J. Portier, J. Bell, M. A. Schexnayder and J. N. Losso. 2003. Biochemical properties of black drum and sheepshead seabream skin collagen. Journal of Agricultural and food Chemistry. 51(27): 8088-8092.

[10] F. Pati, B. Adhikari and S. Dhara. 2010. Isolation and characterization of fish scale collagen of higher thermal stability. Bioresource Technology. 101(10): 3737-3742.

[11] J. W. Woo, S. J. Yu, S. M. Cho, Y. B. Lee and S. B. Kim. 2008. Extraction optimization and properties of collagen from yellow fin tuna (Thunnus albacares) dorsal skin. Food Hydrocolloids. 22(5): 879-887.

[12] Basha SK, Kumar RVS, Haragopal V *et al*. 2011. Effects of fish scales extracted collagen biocastings oncutaneous wound healing in dogs. Res J Pharma BiolChem Sci 2: 36-49.

[13] Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai,T. and Tanaka, M. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeyesnapper (Priacanthus tayenus). Food Chemistry. 89: 363-372.

[14] Lodish H, Berk A, Zipursky SL, *et al.* "Molecular Cell Biology. 4th edition." 4th edition. New York: W. H. Freeman; 2000. Section 22.3, Collagen: The FibrousProteins of the Matrix.

[15] Meena C, Mengi,SA, Deshpandy SG. 1999. Biomedical and industrial applications of collagen. Proc Indian Acad Sci (Chem Sci) 111 (2): 319-329.

[16] Muthukumar T., Senthil R., Sastry T. P. 2012. Synthesis and characterization of biosheet

impregnated with Macrotyloma uniflorum extract for burn / wound dressings. Journal of Colloids and Surfaces B: Biointerfaces.

[17] Mandal, A, 2014. Collagen - What is Collagen? Retrieved from News Medical, url: http://www.news-medical.net/health/Collagen-What-is-Collagen.aspx.

[18] Nagai, T. and Suzuki, N. 2002. Collagen of the skin of ocellate puffer fish (Takifugu rubripes). Food Chemistry, 78, 173-177.

[19] Nalinanon, S., Benjakul, S., Visessanguan, W. and Kishimura, H. 2008. Tuna Pepsin: Characteristics and its use for collagen extraction from the skin of threadfin bream (Nemipterusspp.). Journal of Food Science. 73: C413-C419.

[20] Ogawa, M., Moody, M. W., Portier, R. J., Bell, J., Schexnayder, M. A. and Losso, J. N. 2003. Biochemical properties of black drum and sheeps head sea bream skin collagen. Journal of Agricultural Food Chemistry. 51: 8088-8092

[21] Pati, F.; Adhikari, B.; Dhara, S. Isolation and characterization of fish scale collagen of higher thermal stability. Bioresour. Technol. 2010, 101, 3737–3742.

[22] J.-W. Woo, S. J Yu, S. M. Cho, Y. B. Lee and S. B. Kim. 2008. Extraction optimization and properties of collagen from yellow fin tuna (Thunnus albacares) dorsal skin Food Hydrocolloids. 22:879-887.