PREPARATION OF GELATIN DERIVED FROM FISH SKINS OF FISH SPECIES, CIRRHINUS MRIGALA (NGA-GYIN) AND NOTOPTERUS CHITALA (NGA-PHE) AND THEIR CHARACTERIZATION

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Abstract

The aim of the research work is the isolation of gelatin powder from *Cirrhinus mrigala* (Nga-gyin) and *Notopterus chitala* (Nga-phe) fish skin. Gelatin was derived from the thermal degradation of collagen, which is the principal protein found in skin and bones. Gelatin was extracted by acid treatment and confirmed by FT IR analysis. The physicochemical properties of prepared gelatin such as moisture content, ash content, pH and solubility in water, colour appearance, odour description, viscosity, gelling and melting temperatures were also determined. The yield percent of prepared gelatin from Nga-phe skin was greater than that of Nga-gyin skin. The prepared gelatin was characterized by Fourier Transform Infrared (FT IR) spectrophotometer, Scanning Electron Microscopy (SEM) and Thermogravimetric-Differential Thermal Analysis (TG-DTA). FT IR analysis showed the chemical bond formation of gelatin. Morphological investigation showed that the Nga-gyin skin (Ngy) exhibited sponge or coral structure and the surface of Nga-phe skin (Nph) had denser strand with small pores. TG -DTA data showed three steps of degradation. The initial degradation was due to moisture, the second was due to thermal degradation of composite and the third was denaturing of protein. Therefore, the use of fish skin waste as raw material in the gelatin production is quite potential and plays a major role in recycling of waste.

Keywords: *Cirrhinus mrigala, Notopterus chitala,* gelatin, thermal degradation, collagen, acid treatment

Introduction

Gelatin, a protein with the molecular formula $C_{102}H_{151}N_{31}O_{39,}$ derived from collagen is the major structural protein in connective tissue of animal skin and bone (Cho *et al*., 2004). It is an important constituent in a number of food and non-food products due to its multi-functional properties, thermal stability, digestibility, solubility and its biological characteristics.

In the food industry, it serves primarily as a gelling agent, but it is also used as a thickener, film former, stabilizer, emulsifier, adhesive agent, foaming agent, protective colloid and as a beverage fining agent (Johnston-Barks *et al*., 1990). The quality of gelatin for a particular application therefore depends largely on its rheological properties that are desirable for that application (Gomez- Guillen *et al*., 2002).

Commercially, two main types of gelatin are used: Type A and Type B gelatins (Ward and Courts, 1977). Type A gelatin results from acid process and Type B gelatin results from alkaline process. Dry commercial gelatins for the food industry usually contain about 88% protein, 10% water and 1 – 2% salts (GME, 1990).

Gelatin is being widely used in food, drug and cosmetic industries as stabilizing, thickening and gelling agent (Kittiphattanabawon *et al.*, 2010). The quality of gelatin is largely determined by its gelling strength and thermal stability. This is dependent on the amino acid composition which is species specific and molecular weight distribution as influenced by processing conditions (Gomez-Guillen *et al.*, 2002).

Fish by-products are seldom used as a source of raw materials for gelatin extraction. They are mainly used for animal feed supplements due to their small size. However, some studies have

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ascertained freshwater and marine water fish to have vast amounts of waste after removal of useful edible parts and high gelatin yield being expected from them. Additionally, most findings suggest that gelatin from these species has an advantage over those extracted from cold water species, providing better rheological properties nearly similar to mammalian gelatins.

Fish skin contains large amounts of collagen and can be considered as a potential source of gelatin. One major advantage of gelatin from aquatic sources is that it is not associated with the risk of Bovine Spongiform Encephalopathy and is acceptable to most religious groups. Further, the utilization of fish skin for the extraction of gelatin can significantly address the problem of waste disposal in the fish processing industry. Although fish gelatin will be unable to completely replace mammalian gelatin, in future it might become a niche product offering unique and competitive properties to other biopolymers, as well as meeting the demand of global halal market (Karim and Bhat, 2009).

In the present paper, gelatin was extracted from nga-gyin and nga-phe fish skin used by acid treatment. The physicochemical properties of extracted gelatin were investigated. Moreover, the extracted gelatin was characterized by modern techniques such as FT IR, SEM and TG-DTA analysis.

Materials and Methods

Raw Materials and Chemicals

Nga-gyin (NGY) fish skin and Nga-phe (NPH) fish skin were collected from Hlaing Yadanar Market, Hlaing Township Yangon. Other requiring chemicals were purchased from chemical store. Distilled water was used as the solvent in all analyses.

Extraction of Gelatin

Fish skins stored at -20°C were thawed and cut it into small size of about 1 cm^2. The fish skins were thoroughly rinsed with limewater to remove superfluous materials. The samples (100 g) were rinsed and soaked in 1 % (w/v) citric acid (1:3 w/v) for 12 h. The samples were neutralized by washing several times until the pH of the washing water was faintly at basic pH (pH 6-7).

The fish skins were extracted in distilled water at 60°C for 6 h. The solubilized gelatin was separated from residual skin fragments by filtration through a fabric filter followed by Whatman No. 1. The mixture was cooled until gelatin gel was formed, and then dried using an oven at 60°C for 24 h. The dried gelatin was ground and sieved to produce gelatin powder (Gomez-Guillen *et al*., 2002).

Yield of gelatin

Yield percent of gelatin was calculated by the following equation:

$$\% \text{ yield (wet weight basis)} = \frac{\text{dried weight of gelatin (g)}}{\text{wet weight of fish skin waste (g)}} \times 100$$

Determination of Viscosity

Gelatin solutions at the concentration of 6.67% (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60°C for the determination of viscosity. The viscosity (cP) of 10 mL of the solution was determined using Atago digital viscometer equipped with a No.2 spindle.

Determination of Gelling temperature and Melting temperature

Briefly, 20 mL of gelatin extracts were taken onto test tubes keep in freezer (- 4°C). The gelling temperature was noted. The freezed gelatin kept in water bath at 40°C and the time period corresponding to the melting temperature was recorded.

Characterization of the Prepared Samples

The physicochemical properties (moisture, ash, pH, solubility in water, colour appearance, odour description, viscosity, gelling and melting temperature) of gelatin prepared from nga-gyin (NGY) fish skin gelatin and nga-phe(NPH) fish skin gelatin were determined. The structural characterization of gelatin extracted from NGY and NPH fish skins were characterized using FT IR. The morphological structure of prepared samples were characterized by SEM. Thermal stability of prepared samples were characterized by TG-DTA. FT IR spectrum was recorded in the range of 4000-400 $cm^{-1}$by using 8400 SHIMADZU, Japan FT IR spectrophotometer. The scanning electron microscopy (SEM) images were recorded by using JSM-5610 Model SEM, JEOL-Ltd., Japan. Thermogravimetric analysis of samples were performed using TG-DTA apparatus, (Hi-TGA 2950 model) at the temperature range between 0 and 600°C under nitrogen gas flushing (at a rate of 50 mL/min).

Results and Discussion

Physicochemical Properties of Nga-gyin and Nga-phe gelatin

Table 1 shows the physicochemical properties (moisture, ash, pH and solubility in water, colour appearance, odour description, viscosity, gelling and melting temperature) of gelatin obtained from nga-gyin fish skin and nga-phe fish skin. In this table, nga-gyin gelatin was found to have a significant higher content of moisture and yield percent than those of nga-phe gelatin. The value obtained for the moisture content of gelatin was within the acceptable range (9–12 %) for high quality gelatin giving an indication of good shelf life. The ash content of nga-gyin gelatin and nga-phe gelatin were 2.56 % and 4.88 %, respectively. The value of nga-gyin gelatin is less than the recommended maximum limit of 2.6 % (Jones, 1977) and the limit given for edible gelatin i.e. 2 %. Low ash content suggested that the extracted gelatin was of high quality, as the ash content for a high quality gelatin should be lower than 2 % (Ockerman and Hansen, 1988). The high content of ash for nga-phe gelatin is due to improper washing method during extraction. The pH of nga-gyin gelatin and nga-phe gelatin were 4.21 and 5.48 respectively. pH values are variable and dependent on the gelatin extraction process; thus, different pH ranges can be observed. Jamilah and Harvinder (2002) reported pH values for extracted from the skin of black tilapia (3.81) and red tilapia (3.05). Both of gelatin are soluble in water.

Colour appearance of nga-gyin gelatin and nga-phe gelatin were white and dark yellow colour. Both of gelatin had less fishy odour. Low viscosity of nga-gyin and nga-phe gelatin might be due to over hydrolysis of the collagen during the pretreatment steps. The viscosity of most of the commercial gelatins also have been reported to be in the range of 2.0 to 7.0 cP and upto 13.0 cP for specialized ones (Johnston-Barks *et al*., 1990), Significant differences were observed in the melting temperature of the nga-gyin and nga-phe fish skin gelatin samples. Gelatin with high melting temperature formed stronger gel, and it was also observed that nga-gyin which had higher melting temperature formed stronger gel than nga-phe gelatin. Setting (gelling) temperature denotes the gelling process which involves the transition from random coil to triple helical structure of gelatin.

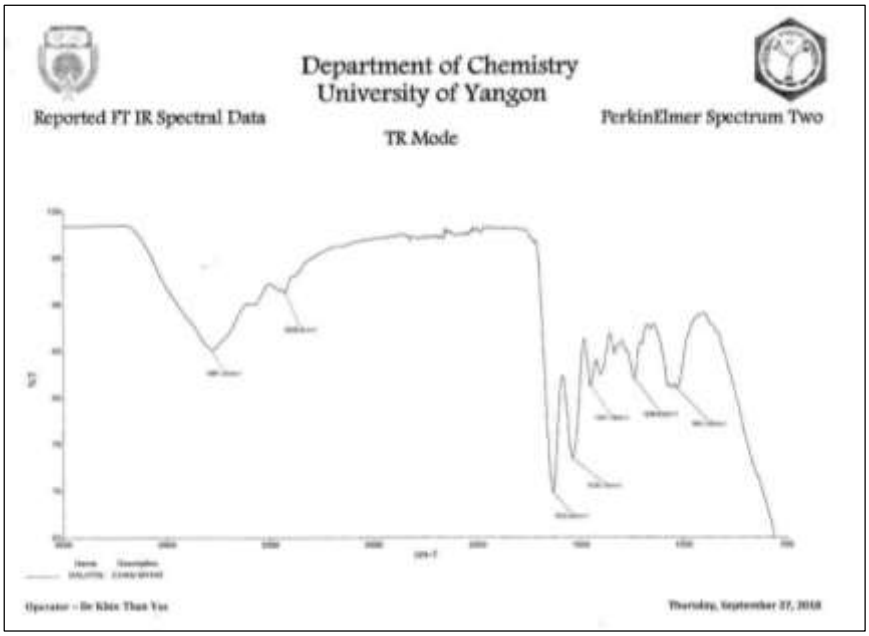
Table 1 Physicochemical Properties of Nga-gyin and Nga-phe gelatin

Physicochemical Properties	NGY-GLT	NPH-GLT
moisture (%)	11.70	5.24
ash (%)	2.56	4.88
pH	4.21	5.48
solubility in water	soluble	soluble
yield percent (%)	10.08	9.18
colour appearance	white	dark yellow
odour description	less fishy odour	less fishy odour
viscosity (cP)	3.8	3.0
gelling temperature (℃)	10	9
melting temperature (℃)	32	23

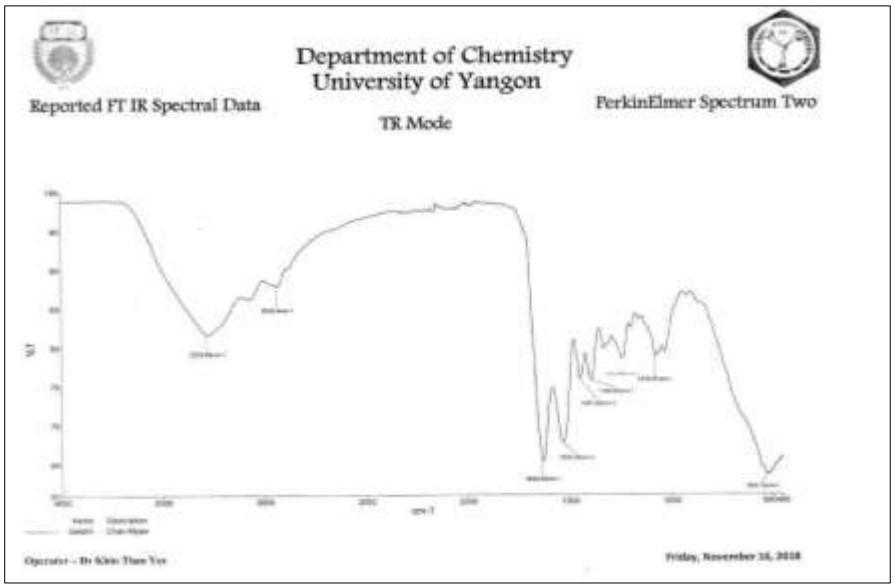
NGY-GLT = Nga-gyin fish skin gelatin
NPH-GLT = Nga-phe fish skin gelatin

FT IR Analysis

FT IR spectroscopy has been used to monitor the functional groups and secondary structure of gelatin. Proteins are comprised of amino acids joined together by amide bonds. The polypeptide and protein repeat units give rise to nine characteristic infrared (IR) absorption bands, namely; amide A, B, and I – VII. Amide bands represent different vibration modes of the peptides bond. The absorption bands of gelatin in the IR spectra are situated in the amide band region; Amide-I represents C=O stretching/hydrogen bonding coupled with COO, Amide-II represents bending vibration of N-H groups and stretching vibrations of C-N groups, Amide-III is related to the vibrations in plane of C-N and N-H groups of bound amide (Nur Hanani *et al*., 2011). Figure 2 shows the result of the FT IR analysis of NGY-GLT and NPH-GLT. Based on the FT IR spectra, the peaks of the gelatin at 3400-3200 cm^{-1} attributed to the presence of hydrogen bond water and amide-A, 1660-1580 cm^{-1} peaks were due to the occurrence of amide-I, 1550-1510 cm^{-1} indicated amide-II, band at 1275-1200 cm^{-1} indicated the amide-III, peaks range from 1460cm^{-1} to1380cm^{-1} were attributed to the symmetric and assymmetric bending vibrations of methyl group.



(a)



(b)

Figure 2 FT IR spectra of prepared (a) nga-gyin skin gelatin powder and (b) nga-phe skin gelatin powder

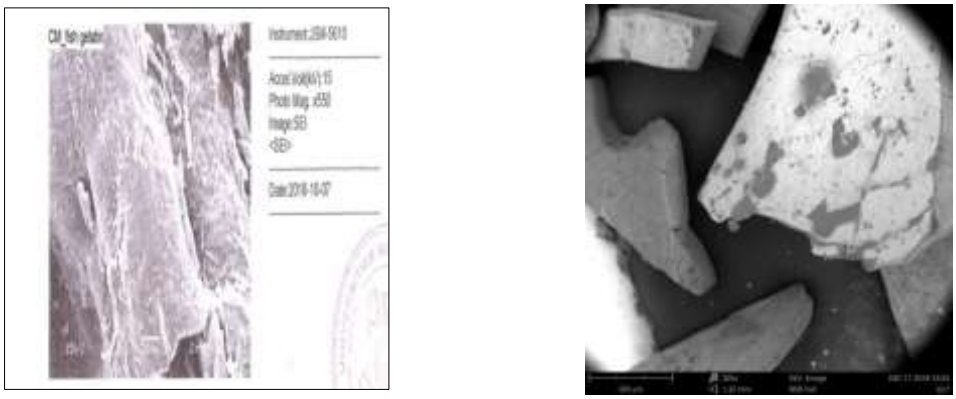
Table 2 FT IR Band Assignments of the Prepared Gelatin from Nga-gyin and Nga-phe Skin Gelatin Powder

Observed wavenumber (cm^{-1})		* Literature wavenumber (cm^{-1})	Band Assignments
Nga-gyin	**Nga-phe**		
3281	3278	3400-3200	O-H stretching
2926	2936	2940-2915	C-H stretching in -CH_2 group
1633	1630	1660-1580	C=O stretching (amide I)
1538	1535	1550-1510	C-N stretching
1451	1451	1485-1455	C-H bending (asym:) in alkane
-	1395	1395-1365	C-H bending (symm:)
1238	1242	1275-1200	C-O-C asym: stretching
1031	1079	1055-1028	C-O stretching

***** Silverstein *et al.*, 2003

SEM Analysis

In order to further investigate the structural changes in the gelatins, SEM micrographs of the NGY-GLT and NPH-GLT are shown in Figure 3. Benjakul *et al* (2009) noted that the arrangement and combination of protein molecules in gel matrix contributes to the gel strength. According to the SEM image, NGY-GLT powder had sponge or coral structure. The structure of the NPH-GLT powder had rather thick, clear and uniform tissue.

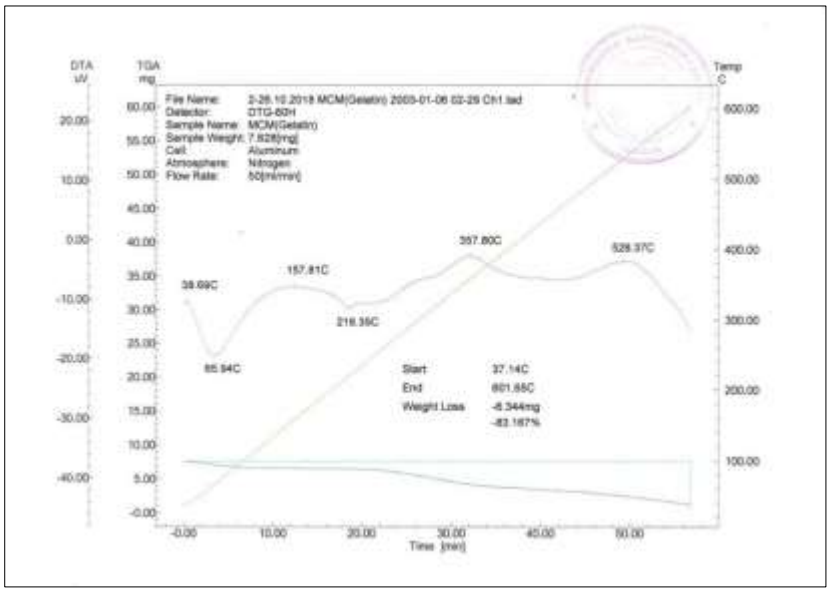


(a) (b)

Figure 3 SEM micrographs of (a) nga-gyin fish skin gelatin (NGY-GLT) and (b) nga-phe fish skin gelatin (NPH-GLT)

TG-DTA Analysis

TG-DTA analysis was carried out by heating the sample at 20°C/min in the temperature range 0-600°C in nitrogen atmosphere and the flow rate 50 mL/min. TG-DTA curve showed the change in mass with the increase of temperature. The results of NGY-GLT and NPH-GLT are shown in Figure 4 and Tables 3 and 4. In this analysis actual weight losses were observed in three steps degradation and the related temperature range was 35-600°C. The initial weight losses were due to the dehydration of adsorbed water and moisture, the second degradation resulted from dehydration of absorbed water and burning of organic compounds in the samples and third losses were denaturing and decomposition of protein in gelatin samples.



(a)

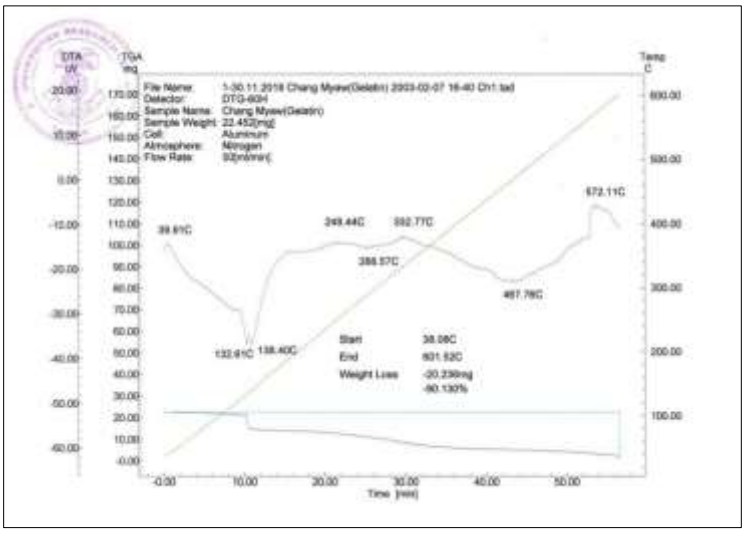


Figure 4 TG-DTA thermograms of prepared gelatin from (a) nga-gyin skin and (b) nga-phe skin

Table 3 TG-DTA Thermogram of Prepared Gelatin from Nga-gyin Skin

Temp: range (ºC)	Weight loss (%)	Peak's Temp: (ºC)	Nature of Peak	TG Remark
37-130	13.66	66	endothermic	due to the dehydration of adsorbed water and moisture
130-365	30.04	357	exothermic	due to the decomposition of volatile materials
365-600	39.47	528	exothermic	due to the decomposition and degradation of gelatin

***** Chen *et al*., 2005

Table 3 (b) TG-DTA Thermogram of Prepared Gelatin from Nga-phe skin

Temp: range (ºC)	Weight loss (%)	Peak's Temp: (ºC)	Nature of Peak	TG Remark
38-130	7.85	133	endothermic	due to the dehydration of adsorbed water and moisture
130-250	37.76	249	exothermic	due to the dehydration of absorbed water and burning of organic compounds
250-600	44.52	333 572	exothermic exothermic	due to the denaturing and decomposition of protein in gelatin

***** Chen *et al*., 2005

Conclusion

In this study, gelatin was extracted from Nga-gyin and Nga-phe by acid treatment. Nga-gyin gelatin was a significant higher content of moisture and yield percent than that of nga-phe gelatin. The value obtained for the moisture content of gelatin was within the acceptable range (9 – 12 %) for high quality gelatin giving an indication of good shelf life. Low ash content suggested that the extracted gelatin was of high quality. The high content of ash for nga-phe gelatin is due to improper washing method during extraction. Significant differences were observed in the

melting temperature of the nga-gyin and nga-phe fish skin gelatin. Nga-gyin which had the higher melting temperature formed the stronger gel than nga-phe gelatin. FT IR spectroscopy has been used to monitor the functional groups and secondary structure of gelatin. The peaks of the gelatin at 1238 cm^{-1} is due to the the presence of functional group (-O- CH_3) and 2926 cm^{-1} is related with the symmetric and asymmetric stretching vibration of the aliphatic group (CH_2). According to the SEM image, NGY-GLT powder had sponge or coral structure. The structure of the NPH-GLT powder had rather thick, clear and uniform tissue. TG-DTA analysis showed three steps degradation due to the dehydration of adsorbed water, burning of organic compounds in the samples and denaturing and decomposition of protein in gelatin samples.

In this study, the physicochemical properties of nga-gyin and nga-phe fish skin gelatin had illustrated the potential of high quality of gelatins that could be used in food applications to replace mammalian gelatin. Therefore, the use of fish skin waste as raw material in the gelatin production is quite potential and plays a major role in recycling of waste.

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