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Effect of extraction time on the physico-chemical characteristics of collagen from sin croaker *(Johniecop sina)* waste

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Abstract

Fish waste is a major by-product of fish processing industries. Sin croaker *(Johniecop sina)* waste could be a valuable source of collagen and utilizing such waste will reduce pollution associated with fish processing industries. The objective of this study is to determine the physico-chemical characteristics of sin croaker waste collagen extracted using 0.5 M acetic acid as a function of extraction time which were 3 and 5 days. The extracted collagens were also compared with the commercial collagen. Results showed that extraction at the duration of 3 and 5 days produced 2.74 and 3.35% yields of collagen, respectively. Both collagens were most soluble at low pH (pH 1-2) and low salt concentration (2% NaCl). Differential Scanning Calorimetry (DSC) analysis showed that the extracted collagen had good thermal stability as it denatured at 31.31°C and 32.10°C, respectively. Commercial collagen denatured at 30.60°C. Sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) indicated that both collagens are of Type I due to the presence of α1, α2 chains, β and γ components. Commercial collagen showed only smeared band. Thus, it is concluded that sin croaker collagens extracted at the duration of 3 and 5 days exhibited similar characteristics and are of equally good quality.

Introduction

Collagen is the most abundant protein of animal origin comprising approximately 30% of the total protein (Pati *et al.*, 2010). Almost all animal parts especially tissues and bones contain collagen which contributes to the stability and maintenance of tissues and organs structural integrity (Nakamura *et al*., 2003; Bae *et al.*, 2008). Collagen is usually extracted using salt, acid or pepsin producing collagens known as the salt, acid or pepsin soluble collagens (Nagai *et al*., 2001; Jongjareonrak *et al*., 2005b; Zhang *et al.,* 2007; Nalinanon *et al*., 2010; Huang *et al.*, 2011; Matmaroh *et al*., 2011). Collagens from marine sources have extensively been studied. These include collagens from the skin, bone and fin of both salt and freshwater fish such as shark skins, brownstripe red snapper skin, skins and bones of bigeye snapper, squid skin, skins of young and adult Nile perch and outer skins of paper nautilus (Kolodziejska *et al*., 1999; Nagai and Suzuki, 2000; Yoshimura *et al*., 2000; Ikoma *et al.*, 2003; Muyonga *et al*., 2004; Jongjareonrak *et al.*, 2005a; Kittiphattanabawon *et al.*, 2005).

Seafood processing discards accounted for approximately three-quarters or as high as 75% of the total weight of the fish (Shahidi, 1994). Some of these wastes are used as animal feed but most are discarded either in-land or hauled into the ocean which could contribute to environmental pollution (Nagai *et al*., 2004; Wooa, 2008). Although mammalian collagens are widely available, its commercialisations are limited due to religious restriction and disease they might transmit. Therefore, more research are necessary to venture into alternative sources of collagen in replacement for mammalian collagen (Shen *et al*., 2007). Sin croaker is one of the several types of fish used in fish processing industries where only the flesh are being used living behind the skin, bones, fin and scales which are usually discarded. Such waste could be a useful source of collagen. Extraction time is one of the important factors in determining the yield and physico-chemical properties of the resulting collagen. Thus, this study was carried out to determine the effect of extraction time on the physico-chemical properties of sin croaker collagen. The extracted collagens were also compared with the commercial collagen.

Materials and Methods

Materials

Sin croaker waste was purchased from Kimi Ocean Food Industries (M) Sdn. Bhd. Kuala Lumpur,

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Malaysia. Commercial collagen was obtained from Toujours Skin Enterprise, Malaysia. The waste was kept at -20°C before used. All reagents used were of analytical grade purchased from Sigma Aldrich, USA.

Preparation of collagen

Non-collagenous proteins were removed by soaking in 0.1M NaOH for 48 hrs at a sample to alkali solution ratio of 1:10 (w/v) according to Duan *et al.* (2009). The alkali solvent was changed every 12 hrs. The residue was washed with cold distilled water to a neutral pH. To extract the collagen, deproteinised bone was decalcified with 0.5 M EDTA-4Na (pH 7.4) using a residue to EDTA-4Na ratio of 1:10 (w/v) for 24 hr. The solution was changed every 12 hrs. The residue was washed thoroughly with cold water (4°C) followed by soaking in 0.5 M acetic acid with a residue to solvent ratio of 1:15 (w/v) for 3 days. Then, the mixture was filtered through two layers of cheesecloth. NaCl was added to a final concentration of 2.6 M. The mixture was centrifuged at 5000 rpm for 30 mins using a centrifuge (Model EV231, Consort, Belgium). The precipitate was then collected and dissolved in 0.5 M acetic acid, dialysed against 0.1 M acetic acid followed by distilled water and then freeze-dried. Similar method was repeated for the extraction time of 5 days.

Collagen yield

Collagen yield was calculated using the following formula based on Muyonga *et al.* (2004):

$$\text{Collagen yield (\%)} = \frac{\text{weight of freeze dried collagen (g)}}{\text{wet weight of fish waste (g)}} \times 100$$

Chemical analysis

Moisture, ash and protein contents were determined according to AOAC (2003). Moisture was determined by moisture analyser (OHAUS Moisture Balance MB45, Switzerland), ash content by igniting the sample in a muffle furnace at 550°C to form a light grey ash. Crude protein content was determined by macro-Kjeldahl method.

Effect of pH on solubility

An amount of 1 g sample was dissolved in 8 ml of 0.5 M acetic acid and transferred to a 50 ml centrifuge tube. The pH was adjusted with 6 M of either NaOH or HCl to obtain the final pH ranging from 1 to 10. The volume of the solution was made up to 10 ml with deionized water. Then, the solution was centrifuged (Centrifuge Model 5420, Kubota Corporation) at 5000 rpm for 30 min at 4°C. Protein concentration in the supernatant was measured using Lowry method (Lowry, 1951). Relative solubility was calculated in comparison with that obtained at the pH exhibiting the highest solubility.

$$\text{Relative solubility (\%)} = \frac{\text{protein content of supernatant}}{\text{total protein content in the sample}} \times 100$$

Effect of NaCl on solubility of collagen

An amount of 1g collagen was mixed with 5 ml NaCl in 0.5 M acetic acid at selected concentrations from 0 to 8% (w/v). The mixture was stirred continuously at 4°C for 30 mins followed by centrifuging at 5000 rpm at 4°C for 15 mins using a centrifuge (Model 5420, Kubota Corporation, Japan). Protein concentration in the supernatant was measured using Lowry method (Lowry, 1951). Relative solubility was calculated in comparison with that obtained at the NaCl concentration exhibiting the highest solubility.

$$\text{Relative solubility (\%)} = \frac{\text{protein content of supernatant}}{\text{total protein content in the sample}} \times 100$$

Viscosity of collagen solution

Viscosity measurement was performed with slight modification according to the method by Normah and Nur-Hani Suryati, (2015). A viscometer (Model LVT Brookfield, USA) with spindle No. 1 and speed of 100 rpm was used. Sample of 0.3% collagen in 0.1 M acetic acid was prepared by dissolving 0.3 g collagen in 100 ml of 0.1 M acetic acid and then heated from 4 to 50°C with a heating rate of 4°C/min. At each designated temperature, the solution was held for 30 min prior to viscosity determination. Measurement was carried out in triplicate. The relative viscosity was calculated in comparison to that obtained at 4°C.

Differential scanning calorimetry

Maximum transition temperature (T_{max}) was determined by method of Rochdi *et al.* (2000) and Komsa-Penkova *et al.* (1999). The sample was rehydrated by adding 0.05M acetic acid at a solid/solution ratio of 1:10 (w/v). The mixture was allowed to stand for 2 days at 4°C. The samples (10 mg) was accurately weighed into aluminium pans, sealed and then scanned through the Differential Scanning Calorimetry (Diamond DSC, Perkin Elmer, USA) at 2°C/min over the range of 20 to 50°C. An empty pan was used as reference. Temperature calibration was done using Indium. Total denaturation enthalpy was estimated by measuring the area in the DSC thermogram. The maximum transition temperature (T_{max}) was estimated from the thermogram.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoretic patterns of the collagen were visualized by SDS-PAGE comprising of 7.5% resolving gel and 4% stacking gel. Collagen sample was suspended in 5% (w/v) SDS prior to incubation at 85°C for 1 hr. The mixture was centrifuged in a centrifuge (Model 5420, Kubota Corporation, Japan) at 5000 rpm for 10 mins at room temperature to remove undissolved debris. Collagen was mixed with sample loading buffer (60 mM TrisHCl, pH 8.0, containing 25% glycerol, 2% SDS and 0.1% bromophenol blue) at the ratio of 4:1 (w/v) according to Normah *et al.* (2005). An amount of 20 µl sample was loaded into each well and then run in an electrophoresis instrument (Model BIOFUGE pico, Heraeus, Germany). The electrophoresis was carried out for about 4 hrs at a constant voltage of 100 V. After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie blue R-250 in 45% (v/v) methanol and 10% (v/v) acetic acid. High molecular weight protein marker ranging from 10 to 220 kDa was used to estimate the molecular weight of the proteins. Commercial collagen was used for comparison.

Statistical analysis

All experiments were run in triplicates. Statistical analyses were conducted with the Statistical Analysis System (SAS) (SAS Institute Inc., 2004). Analyses of variance were performed by ANOVA procedures. Significant differences at $p < 0.05$ were used to identify differences between means.

Results and Discussion

Yield

The waste used in this study for the production of collagen comprises of bones and scales mixture with very small proportion of the skin. The yields of collagen obtained from the 3 and 5 days of extraction were 2.74 and 3.35%, respectively (Table 1). Extending the extraction time to 5 days resulted in significantly ($p<0.05$) higher yield. Previously, yields of collagen from bigeye snapper bone produced using 0.5M acetic acid was 1.6% while the yields from silver carp and spotted goatfish scales were 1.45 and 0.46%, respectively (Kittiphattanabawon *et al*., 2005; Zhang *et al.*, 2010; Matmaroh *et al*., 2011). Similarly, when collagen was separately extracted from the skin, bones and scales using 0.5M acetic acid at the duration of 3 to 4 days, collagen from the skin recorded higher yield which was 41.3% while extraction from bones and scales resulted in only 1.35% and 1.06%, respectively (Duan *et al.*, 2009). The concentrations of acetic acid influence the extractability of collagen (Sadowska *et al.*, 2003; Wang *et al*., 2008). Previous study showed that higher yield was achieved at 0.5M acetic acid compared to 0.8M (pH 2.4) where at low pH, collagen denatures (Wang *et al*., 2008). Higher yield at 0.5M acetic acid was also attributed to the presence of more positively charged amine groups of collagen at this concentration (Wang *et al*., 2008). In another study, 0.46% yield was obtained for acid soluble collagen compared to 1.20% for pepsin soluble collagen from the scale of spotted golden goatfish (Matmaroh *et al*., 2011). Higher yield for bone collagen from different fish species was obtained although similar extraction solution and time were used (Nagai and Suzuki, 2000). The yields were 42.3% from skipjack tuna, 40.7% from Japanese sea-bass, 53.6% from ayu, 40.1% from yellow sea bream and 43.5% from horse mackerel, respectively. Difference in yield could be attributed to structural difference of bone collagens between different fish species (Duan *et al*., 2009).

Table 1. Yield, moisture, protein and ash content of commercial collagen and sin croaker waste collagen extracted for 3 and 5 days.

Chemical analysis (%)	Commercial collagen	3 days collagen	5 days collagen
Yield	-	2.74^{b}	3.35^{a}
Moisture	3.12^{a}±0.57	0.44^{b}±0.01	0.49^{b}±0.01
Protein	21.57^{b}±0.88	23.17^{b}±0.09	32.29^{a}±0.07
Ash	2.65^{a}±0.06	1.91^{b}±0.03	1.94^{b}±0.02

Means ± standard deviation of triplicate determinations. Means within row with same lower case are not significantly different ($p < 0.05$).

Chemical analysis

Sin croaker collagens contain 0.44 and 0.49% moisture, 23.17 and 32.29% protein, respectively for 3 and 5 days of extraction (Table 1). Moisture content in collagen from shark skin and nile perch skin were approximately 7.77 and 8.09%, respectively (Muyonga *et al*., 2004; Kittiphattanabawon *et al*., 2010). The collagen extracted from the scales of spotted golden goatfish, silver carp and brownbanded bamboo shark contain 37.91%, 43.43% and 89% protein, respectively (Kittiphattanabawon *et al*., 2010; Zhang *et al.*, 2010; Matmaroh *et al.*, 2011). A positive relationship was observed between the hydroxyproline and protein content which also varies with the fish habitat (Zhang *et al.*, 2010).

The ash contents for 3 and 5 days extracted

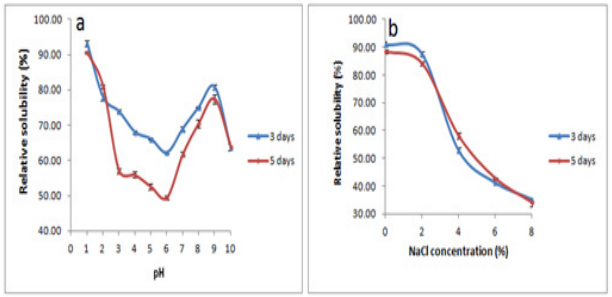

Figure 1. Relative solubility (%) of sin croaker waste collagen extracted for 3 days (—▲—) and 5 days (—■—) as affected by different a) pH and b) concentrations of NaCl.

collagen were 1.91 and 1.94%, respectively which indicated that approximately 98% of inorganic matters were removed. Demineralization step using EDTA during the extraction process might cause looser matrix of the bone and scale which could allow easier extraction of the collagen (Matmaroh *et al*., 2011). Variation in the composition of waste between fish species might have an effect on collagen extraction (Kittiphattanabawon *et al.*, 2010).

Effect of pH on solubility of collagen

Solubility of sin croaker waste collagen as influenced by different pH was studied. Both collagens showed similar trend in solubility at different pH (Figure 1a). High solubility was observed in very acidic pH (1 to 2) and at alkaline pH 9. Solubility was highest at pH 1 which is 94.53% for collagen extracted for 3 days and 89.77% for collagen extracted for 5 days. Similar result was reported for collagen from snakehead *(Ophiocephalus argus)* scale, brownstripe red snapper *(Lutjanus vitta)* and bigeye snapper *(Priacanthus macracanthus)* where higher solubility were recorded in the pH range of 1 to 4 and pH 8 to 10 (Jongjareonrak *et al*., 2005b; Liu *et al*., 2010). Collagen had isoelectric points ranging from pH 6 to 7 (Foegeding *et al.*, 1996). A protein dissolved in buffer at its isoelectric point has no net charge and thus, hydrophobic-hydrophobic interaction increases and protein precipitation as well as aggregation are induced (Liu *et al*., 2010). However, at pH lower or higher than the isoelectric point, the net negative or positive charge residues of protein molecules increases and the solubility is increased by the repulsive force between chains (Vojdani, 1996). Collagen solubility is also influenced by its conformations and molecular properties (Kittiphattanabawon *et al*., 2005).

Effect of NaCl on the solubility of collagen.

The effect of different concentrations of NaCl on the solubility of sin croaker collagen is shown in Figure 1b. There was no significant difference ($p > 0.05$) in solubility for collagen extracted at both

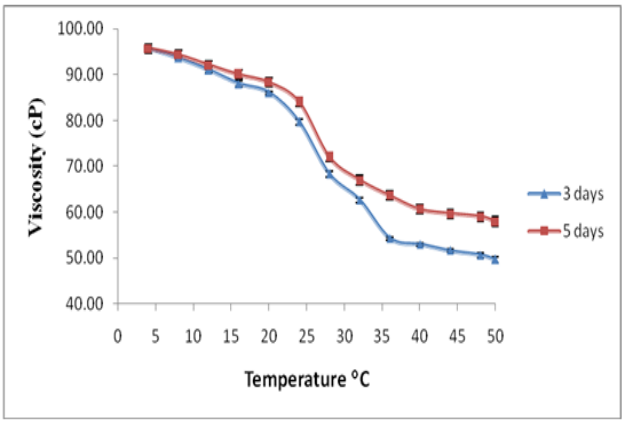

Figure 2. Viscosity (cP) of sin croaker waste collagen extracted for 3 days (—▲—) and 5 days (—■—).

times where solubility decreased in similar trend with increased in NaCl concentration. Collagen solubility remained constant in the presence of NaCl up to 2% (w/v) and sharply decreased beyond 2% NaCl. At zero concentrations, solubilities were 91.03 and 88.38% for 3 and 5 days extraction, respectively. This observation was in accordance with previous studies where the solubilities of various collagens decreased with increase in NaCl concentration (Jongjareonrak *et al*., 2005b; Kittiphattanabawon *et al*., 2005; Bae *et al.*, 2008; Singh *et al.,* 2011). An increase in NaCl concentration could result in declining protein solubility by enhancing hydrophobic-hydrophobic interactions, aggregation between chains and the competing for water of ionic salts, thereby causing the protein to precipitate (Jongjareonrak *et al*., 2005a; Bae *et al*., 2008).

Viscosity of collagen

Changes in viscosity upon heating are shown in Figure 2. Viscosity decreased continuously with increase in heating temperature. Both collagens showed similar changes in viscosity. Viscosity decreased gradually beginning from 20 until 35°C after which it continues to decrease very slowly between 36 to 50°C. Collagen extracted for 5 days was significantly ($p<0.05$) more viscous than the 3 days collagen. High viscosity is one of the physico-chemical characteristics of collagen and can be accounted for by the high proportion of ß and γ chains resulting in a higher average molecular weight (Zhong *et al.*, 2013). For sin croaker collagen, the bands representing the ß and γ chains were broader in the collagen extracted for 5 days than those of 3 days which suggest the slightly higher viscosity values of the 5 days extracted collagen (Figure 4). Furthermore, the solubility of collagen in acetic acid can affect collagen extractability (Wang *et al*., 2008). Extending the exposure time to 5 days caused more

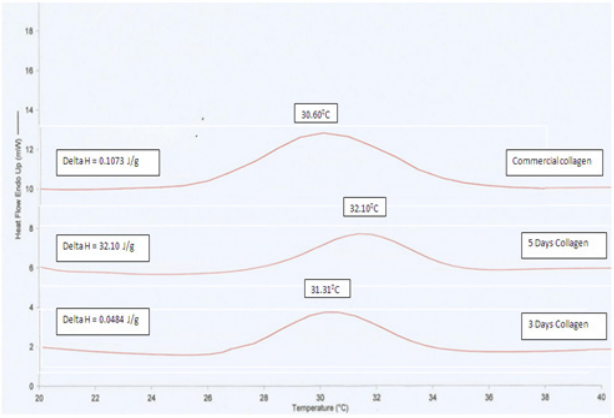

Figure 3. Thermal transition curves of sin croaker waste collagen and commercial collagen.

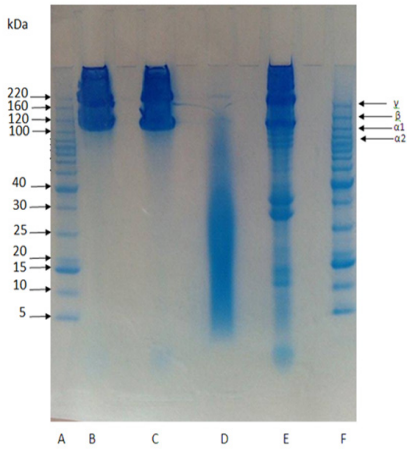

Figure 4. Electrophoretic profile of sin croaker waste and collagens extracted at 3 and 5 days. A and F: protein marker, B: sin croaker collagen (5 days), C: sin croaker collagen (3 days), D: commercial collagen, E: sin croaker waste (bones and scale).

collagen to be solubilised in the acetic acid solution during the extraction process. This leads to higher yield of collagen and higher viscosity readings.

Maximum transition temperature (T_{max})

Differential scanning calorimetry thermogram of sin croaker collagen is shown in Figure 3. The endothermic peaks with maximum transition temperature (T_{max}) for 3 and 5 days extraction were 31.31 and 32.10°C, respectively which is slightly lower than that of threadfin bream skin collagen (33.35°C) (Kittiphattanabawon *et al*., 2005; Nalinanon *et al*., 2011). Commercial collagen has maximum transition temperature (T_{max}) at 30.60°C. Thermal stability of collagen is governed by the pyrrolidine rings of proline and hydroxyproline and partially by hydrogen bonding through the hydroxyl group of hydroxyproline (Benjakul *et al*., 2010). The higher the imino acids content, the more thermal stable is the collagen (Zhang *et al*., 2007). Thermal transition or denaturation temperatures depend on fish species, habitat temperature where the fish lives, seasons and age (Muyonga *et al*., 2004; Duan *et al*., 2009). Different denaturation temperatures (Td) have been reported in collagen from different sources such as from acid soluble collagen of rohu and catla scale (36.5°C), scale and bone of carp (26°C), eagle ray (34.1°C) and yantai stingray (32.2°C) (Bae *et al*., 2008; Duan *et al*., 2009; Pati *et al*., 2010).

Molecular weight distribution

The molecular weight of sin croaker waste vary from lower than 5 to 220 kDa (Figure 4). Collagen from both extraction times showed that the bands ranged from 100 to 220 kDa. No differences in electrophoretic patterns between collagens from both extraction times were observed. Commercial collagen showed smeared band. Type I collagen consists of an identical α1 chains and α2 chain (Pearson and Young, 1989; Wong, 1989; Foegeding *et al*., 1996; Burghagen, 1999). Fish scale and bone have been reported to contain Type I collagen as the major collagen (Kimura and Ohno, 1987; Montero *et al*., 1990; Ciarlo *et al*., 1997; Nagai and Suzuki, 2000). Based on the molecular weight range which is between 100 to 120 kDa where these bands represent the α1 and α2 chains, collagens from sin croaker waste was considered as Type I. The collagen from scales of different species including snakehead fish, sardine, red seabream, Japanese seabass and black drum have also been classified as Type I (Nagai *et al.,* 2004; Ogawa *et al.*, 2004; Liu *et al.,* 2010).

Conclusion

Collagen from sin croaker waste were extracted and characterized. The characteristics of the extracted collagen were compared with the commercial collagen. The extracted collagens showed the characteristics of a Type I collagen with thermal transition temperature close to the commercial collagen. Collagen extracted at 3 and 5 days exhibited similar characteristics except for viscosity where extraction for 5 days produced more viscous collagen. Although the yield was very low, results suggested that sin croaker waste can be used as a source for collagen production.

References

Association of Analytical Chemists (AOAC). 2003. Official Methods of Analysis of AOAC International. 17th ed. Gaithersburg, M.D: Association of Analytical Chemists. Inc.

Bae, I., Osatomi, K., Yoshida, A., Osako, K., Yamaguchi, A. and Hara, K. 2008. Biochemical properties of

acid-soluble collagens extracted from the skins of underutilised fishes. Food Chemistry 108: 49-54.

Benjakul, S., Thiansilakul, Y., Visessanguan, W., Roytrakul, S., Kishimura, H. and Prodpran, T. 2010. Extraction and characterisation of pepsin solubilised collagens from the skin of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*). Journal of the Science Food and Agriculture 90: 132-138.

Burghagen, M. 1999. Collagen. In Belitz, H. D. and Grosch, W. (Eds). Food Chemistry, p. 540-547. Berlin: Springer.

Ciarlo, A. S., Paredi, M. E. and Fraga, A. N. 1997. Isolation of soluble collagen from hake skin *(Merluccius hubbsi)*. Journal of Aquatic Food Product Technology 6 (1): 65-77.

Duan, R., Zhang, J. J., Du, X. Q., Yao, X. C. and Konno, K. 2009. Properties of collagen from skin, scale and bone of carp *(Cyprinus carpio)*. Food Chemistry 112: 702-706.

Foegeding, E., Lanier, T. C. and Hultin, H. O. 1996. Collagen In Fennema, O. R. (Ed.). Food Chemistry 3: 902-906.

Huang, Y. R., Shiau C. Y., Shiau C. Y., Chen H. H. and Huang, B. C. 2011. Isolation and characterization of acid and pepsin-solubilized collagens from the skin of balloon fish *(Diodon holocanthus)*. Food Hydrocolloids 25: 1507-1513.

Ikoma, K., Kobayashi, H., Tanaka, J., Walsh, D. and Mann, S. 2003. Physical properties of type I collagen extracted from fish scales of Pagrus major and Oreochromis niloticas. Food Chemistry 32:199-204.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Nagai T. and Tanaka, M. 2005a. Isolation and characterisation of collagen from bigeye snapper *(Priacanthus marcracanthus)* skin. Journal of the Science of Food and Agriculture 85: 1203-1210.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Nagai, T. and Tanaka, M. 2005b. Isolation and characterization of acid and pepsin-solubilised collagens from the skin of brownstripe red snapper *(Lutjanus vitta)*. Food Chemistry 93: 475-484.

Kimura, S. and Ohno Y. 1987. Fish type I collagen: Tissue specific existence of two molecular forms, α1 α2 α3, in Alaska pollack. Comparative Biochemistry and Physiology Part B 88: 409-413.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Kishimura, H. and Shahidi, F. 2010. Isolation and characterisation of collagen from the skin of brown bandedbamboo shark *(Chiloscyllium punctatum)*. Food Chemistry 119: 1519-1526.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T. and Tanaka, M. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper *(Priacanthus tayenus)*. Food Chemistry 89: 363-37.

Kolodziejska, I., Sikorski, Z. E. and Niecikowska, C. 1999. Parameters affecting the isolation of collagen from squid *(Illex argentinus)* skins. Food Chemistry 66: 153–157.

Komsa-Penkova, R., Koyonava, R., Kostov, G. and Tenchov, B. 1999. Discrete reduction of type I collagen thermal stability upon oxidation. Biophysical Chemistry 83: 185-195.

Liu, Z., Oliveira, A. C. M. and Su, Y.C. 2010. Purification and characterization of pepsin-solubilised collagen from skin and connective tissue of giant red sea cucumber *(Parastichopus californicus)*. Journal of Agricultural and Food Chemistry 58: 1270-1274.

Lowry, O.H., Rosenbrough, N. N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Matmaroh, K., Benjakul, S., Prodpran, T., Encarnacion, A. B. and Kishimura, H. 2011. Characteristic of acid soluble collagen and pepsin soluble collagen from the scale of spotted golden goatfsh *(Parupeneus heptacanthus)*. Food Chemistry 129: 1179-1186.

Montero, P., Borderias J., Turnay J. and Leyzarbe M. A. 1990. Characterization of hake (Merluccius L.) and trout (*Salmo irideus* Gibb) collagen. Journal of Agricultural Science and Food Chemistry 38: 604-609.

Muyonga, J. H., Cole, C. G. and Duodu, K. G. 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch *(Lates niloticus)*. Food Chemistry 85: 81-89.

Nagai, T., Izumi M. and Ishii M. 2004. Fish scale collagen. Preparation and partial characterization. International Journal of Food Science and Technology 39: 239-244.

Nagai, T. and Suzuki, N. 2000. Preparation and characterization of several fish bone collagens. Journal of Food Biochemistry 24: 427-436.

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

Nakamura, Y. N., Iwamoto, H., Ono, Y., Nishimura, S. and Tabata, S. 2003. Relationship among collagen amount, distribution and architecture in the *M. longissimus* thoracis and M. pectoralis profundus from pigs. Meat Science 64: 43-50.

Nalinanon, S., Benjakul, S. and Kishimura, H. and Osako, K. 2010. Characterization of collagen from the skin of unicorn leatherjacket *(Aluterus monoceros)* extracted with the aid of albacore tuna pepsin. Journal of Industrial Engineering 3: 53-70.

Nalinanon, S., Benjakul, S. and Kishimura, H. and Osako, K. 2011. Type I collagen from the skin of ornate threadfin bream *(Nemipterus hexodon)* In Characteristic and effect of pepsin hydrolysis. Food Chemistry 125: 500-507.

Normah, I. and Nur-Hani Suryati, M.Z. 2015. Isolation of collagen from the scale and fin of threadfin bream *(Nemipterus japonicus)*. International Food Research Journal 22(6): 2294-2301.

Normah, I, Jamilah B., Nazamid, S. and Yaakub, C.M. 2005. Optimization of the hydrolysis conditions for the production of threadfin bream *(Nemipterus japonicus)* hydrolysate by alcalase. Journal of Muscle

Foods 16: 87-102.

Ogawa, M., Portier, R. J., Moody, M. W., Bell, J., Schexnayder, M. A. and 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.

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

Pearson, A. M. and Young, R. B. 1989. Muscle and meat biochemistry. San Diego: Academic Press Inc.

Rochdi, A., Foucat, L. and Renou, J. 2000. NMR and DSC studies during thermal denaturation of collagen. Food Chemistry 69: 295-299.

Sadowska, M., Kolodziejska, I. and Niecikowska, C. 2003. Isolation of collagen from the skins of Baltic cod *(Gadus morhua)*. Food Chemistry 81(2): 257-262.

SAS Institute 2004. SAS user's guide to the statistical analysis system. Raleigh, NC: SAS Institute Inc.

Shahidi, F. 1994. Seafood processing by-products. In Shahidi, F.and Botta, J. R. (Eds.). Seafoods chemistry, processing, technology and quality, p. 320-334. Glasgow: Blackie Academic and Professional.

Shen, X. R., Kurihara, H. and Takahashi, K. 2007. Characterization of molecular species of collagen in scallop mantle. Food Chemistry 102 (4): 1187-1191.

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

Vojdani, F. 1996. Solubility. In Hall, G.M. (Ed). Method of testing protein functionality, p. 11-60. Great Britain: St. Edmundsbury Press.

Wang, L., Yang, B., Du, X., Yang, Y. and Liu, J. 2008. Optimization of conditions for extraction of acid-soluble collagen from grass carp *(Ctenopharyngodon idella)* by response surface methodology. Innovative Food Science and Emerging Technologies 9(4): 604-607.

Wong, D. W. S. 1989. Mechanism and theory in food chemistry. New York: Van Nostrand Reinhold Company Incorporation.

Wooa, J.W., Yua, S.J., Chob, S.M., Leea, Y.B. and Kima, S.B. 2008. Extraction optimization and properties of collagen from yellowfin tuna *(Thunnus albacares)* dorsal skin. Food Hydrocolloids 22: 879-887.

Yoshimura, K., Terashima, M., Hozan, D. and Shirai, K. 2000. Preparation and dynamic viscoelasticity characterization of alkali-solubilized collagen from shark skin. Journal of Agriculture and Food Chemistry 48: 685-690.

Zhang, J., Duan, R., Ye, C. and Konno, K. 2010. Isolation and characterization of collagens from scale of silver carp *(Hypophthalmichthys molitrix)*. Journal of Food Biochemistry 34: 1343-1354.

Zhang, Y., Liu, W. and Li, G. 2007 Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp *(Ctenopharyngodon idella)*. Food Chemistry 103: 906-912.

Zhong, R. L., Bin W., Chang F. C., Qi H. Z., Yan D.G., Jia J.T., Hong Y.L. and Guo F.D. 2013. Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel *(Scomberomorous niphonius)*. Journal of Food Hydrocolloids 31:103-113.