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Extraction and characterization of pepsin-solubilized collagen from the body wall of crown-of-thorns Starfish (*Acanthaster planci*)

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Abstract

Pepsin-solubilized collagen (PSC) was extracted from the body wall of crown-of-thorns starfish (COTS) (*Acanthaster planci*) using pepsin digestion in 0.5 M acetic acid. The electrophoretic pattern of PSC showed that it consisted of two α chains (α1 and α2 chains). In addition, the peptide mapping showed that there were some differences in peptide patterns among PSC, calf skin collagen and salmon skin collagen. This suggested that the primary structure of the PSC was different from calf skin collagen and salmon skin collagen. Furthermore, Fourier transform infrared spectroscopy (FTIR) investigation showed the existence of triple helical structure in PSC, suggesting that the triple helical structure was well preserved during the extraction of collagen from COTS. The denaturation temperature of PSC was 33.0°C, which was comparable with mammalian collagen. In addition, the amino acid composition analysis showed that the imino acid content of PSC was similar to mammalian collagen but it was higher than other marine collagen. This suggested that the imino acid content might contributed the denaturation temperature of collagen. The results in this study suggest that PSC from the underutilized COTS has potential to be exploited in various commercial applications.

Introduction

Collagen is the most abundant extracellular matrix found in animals, accounting for 30% of the total protein in animal body (Muyonga *et al*., 2004). It is a large protein molecule which composed of three polypeptide chains intertwined to form right-handed triple helical structure. Each polypeptide chain (α chain) consists of a repeating amino acid sequence of Gly-X-Y, where most of the X and Y are proline and hydroxyproline, respectively (Ricard-Blum *et al.*, 2005). Collagen is the structural element for skin, cartilage, bone and other organs in vertebrates whereas in invertebrate, collagen is mainly found in their body walls and cuticles (Conand and Sloan, 1989).

Collagen has been widely exploited as the material in food, cosmetic and biomedical industries due to its low immunogenicity and high biocompatibility (Zhang *et al*., 2007). At present, collagen for industrial purpose is mainly extracted from bovine and porcine origins (Jongjareonrak *et al.*, 2005). However, the outbreak of mad cow disease and foot-and-mouth disease have resulted in rising concerns regarding bovine and porcine collagen as a potential transmitting pathogenic vector of these diseases (Zhang *et al.*, 2007). Therefore, the increasing anxieties about the consumption of collagen or collagen-derived products from terrestrial domestic animals such as cows and pigs have led to extensive study on marine collagen as an alternative to the mammalian collagen. However, most of the present researches on marine collagen are limited to the extraction of collagen from fish (Rigby, 1968; Muyonga *et al*., 2004; Jongjareonrak *et al*., 2005; Zhang *et al.*, 2007; Wang *et al.*, 2008; Kittiphattanabawon *et al*., 2010). Little is known of collagen from other source of marine organisms especially the starfish (Nagai and Suzuki, 2000; Nagai *et al*., 2000; Saito *et al*., 2002; Cui *et al*., 2007).

Crown-of-thorns starfish (COTS) (*Acanthaster planci*) is the second largest starfish, which feeds on live corals. It can be found throughout the Pacific Ocean and Indian Ocean. The population outbreak of COTS have resulted in many corals have been eaten and destroyed. This gives a negative impact on the ecosystem of the coastal region as well as substantial loss to the tourism industry (Brodie *et al.*, 2005). In

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Pulau Tioman and Pulau Redang (at the east coast of Peninsular Malaysia), the COTS are removed from the sea and buried ashore in order to control the population outbreak of the starfish (Mstar Online, 2011). However, this approach may cause offensive odor in the area where these starfishes were buried. Based on literature, the body wall of COTS is mainly composed of collagen (O'Neill, 1989). Therefore, it is possible to extract substantial amounts of collagen from the underutilized COTS. This would provide another potential alternative source of collagen. As the first stage to elucidate this idea, this study was aimed to extract and characterize the collagen from COTS. It is anticipated that the data collected in this study could provide some insights on the physico-chemical properties of collagen from COTS.

Materials and Methods

Chemicals

Type I collagen from salmon skin and lysyl endopeptidase (EC 3.4.21.50) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Type I collagen from calf skin, pepsin (EC 3.4.23.1; 656 units/mg protein), and 2-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Pre-stained SDS-PAGE standards (204 kDa-6.6 kDa), Laemmli sample buffer and Coomassie blue R-250 were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, U.S.A.). All the chemicals used in this study were of analytical grade.

Materials

Crown-of-thorns starfish (COTS) (*Acanthaster planci*) was collected at Pasir Akar and Pulau Lang (at the east coast of Peninsular Malaysia). A voucher specimen for the COTS (registered as number PPSK/USM/CTI-0-07-2009-APLC) was deposited in Centre for Sea Cucumber Research, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. The COTS was cooled in ice and transported to the laboratory. The spines of COTS were first removed. Subsequently, the body wall was dissected and washed thoroughly with distilled water to remove the adherent tissue before it was cut into smaller pieces. The body wall of COTS was then stored at -80°C prior to extraction.

Determination of moisture content

The moisture content of the body wall of COTS was determined by freeze drying the body wall. The moisture content was calculated according to the following equation:

$$\text{Moisture content} = [(w_1 - w_2)/w1] \times 100\%,$$

where w_1 is the weight of the body wall before freeze-drying and w_2 is the weight of the body wall after freeze-drying.

Extraction of pepsin-solubilized collagen (PSC)

The extraction of PSC was performed according to the method of Nagai and Suzuki (2000) with slight modification. The extraction was performed at 4°C. The body wall of COTS was soaked in 0.1M NaOH at a sample-to-solution ratio of 1:10 (w/v) for three days to remove the non-collagenous materials. The insoluble body wall tissue was collected and washed with distilled water. The body wall was then demineralized with 0.05 M Tris-HCl (pH 7.5) containing 0.5 M ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) for 2 days and disaggregated with 0.1 M Tris-HCl (pH 8.0) containing 0.05 M EDTA-2Na, 0.5M NaCl and 0.2M 2-mercaptoethanol for 3 days. The mixture was centrifuged at 3200 g for 30 min in order to collect the collagen fibrils. The collagen fibril was then washed with distilled water for 2 days by changing the distilled water every 24 h.

The collagen fibril was suspended in 0.5 M acetic acid at a sample-to-solution ratio of 1:10 (w/v) for 2 days. The mixture was then centrifuged at 20000x g for 1 h. The undissolved residue was collected. The residue was then re-suspended in 0.5M acetic acid again and digested with pepsin at pepsin-to-collagen fibril ratio of 1:15 (w/w) for 48 h. The resultant viscous solution was centrifuged at 20000x g for 1 h. The resultant supernatant was then dialyzed against 0.02 M Na_2HPO_4 (pH 7.2) for 3 days by changing the solution daily. The dialysate obtained was centrifuged at 20000x g for 1 h. The precipitate obtained was dissolved in 0.5M acetic acid. Salting out was then performed by adding NaCl to the mixture until the NaCl concentration reached 0.9 M. The resultant precipitate was collected by centrifuging the mixture at 20000x g for 1 h. The residue was further precipitated by adding NaCl until the concentration of NaCl reached 2.5M in 0.05 M Tris-HCl (pH 7.5). The mixture was then centrifuged at 20000x g for 1 h and the resultant precipitate was re-dissolved in 0.5M acetic acid before it was dialyzed against 0.1M acetic acid and distilled water, respectively. The dialysate was then subjected to lyophilization. The collagen obtained was referred to as pepsin-solubilized collagen (PSC).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the methods of Laemmli (1970) in order to investigate the electrophoretic pattern of PSC. The SDS-PAGE was carried out using discontinuous Tris-glycine

buffer with 4% stacking gel and 4% resolving gel. PSC, type I collagen from calf skin and type I collagen from salmon skin were dissolved in 0.02 M sodium phosphate (pH 7.2) containing 1% SDS and 3.5 M urea. Subsequently, the dissolved collagen samples were mixed with Laemmli sample buffer containing 5% of 2-mercaptoethanol at the ratio of 1:1 before subjected to SDS-PAGE. A total of 30 µg of each collagen sample was loaded on the gel. The samples were run at 80V for the first 10 min and then run at 120 V until the solvent front reach about 0.5 cm from the end of the gel. After SDS-PAGE, the gel was stained with 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol and 10% (v/v) acetic acid for 2 h. The gel was then destained with destaining solution containing 40% (v/v) methanol and 10% (v/v) acetic acid. Pre-stained SDS-PAGE standards (broad range) were used to estimate the molecular weight of the collagen. Type I collagen from calf skin and salmon skin were used as the standard collagens. The image of the SDS-PAGE gel was captured using FUJIFILM Luminescent Image Analyzer LAS-3000 (Fujifilm, Tokyo, Japan). Multi Gauge version 3.0 software (Fujifilm, Tokyo, Japan) was used to analyze the electrophorestic pattern of the PSC.

Amino acid composition of PSC

A total of 100 mg of dried PSC was mixed with 5 ml of 6M HCl. The mixture was purged with nitrogen gas and vacuum-sealed before heating at 110°C for 24 h. After the hydrolysis, the hydrolysate was added to 400 µl of 50 µmole/ml of L-α-amino-n-butyric acid (AABA) (internal standard). The mixture was then topped-up to 100 ml with deionized water. The sample was then filtered by 0.22 µm membrane filter. Subsequently, the sample was derivatized by adding 10 µl of the sample to 20 µl of AccQ•fluor™ reagent (AQC: 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The mixture was incubated at room temperature for 1 min to allow the reaction to take place. The sample was then heated at 55°C for 10 min before amino acid analysis. A total of 10 µl of sample was injected into the column and the elution was started with a flow rate of 1 ml/min.

The amino acid analysis of PSC was performed using Waters-High Performance Liquid Chromatography (HPLC) system (Milford, MA, U.S.A.) with fluorescence detector. The column used in the analysis was Waters AccQ•Tag™ Amino acid Analysis Column (silica based bonded with C18) (3.9 mm 150 mm). The column was maintained at 37°C during the analysis. Fluorescence was measured at 250 nm for excitation and 395 nm for emission. Identification and quantification of amino acid was performed by comparing the retention time and area with the amino acid standards. The amino acid analysis was carried out in three replicates. The glutamine content was estimated from the glutamic content. Methionine and cysteine was not determined in the analysis of amino acid composition.

Peptide mapping of PSC

Peptide mapping of PSC was performed according to the methods of Jongjareonrak *et al.* (2005). A total of 2 mg of PSC, type I collagen from calf skin and type I collagen from salmon skin were dissolved in 500 µl of 0.1M sodium phosphate (pH 7.2) containing 0.5% SDS, respectively. The digestion was performed by adding 0.05 µg of lysyl endopeptidase to 100 µl of the collagen solution containing 400 µg of PSC. The mixture was incubated at 37°C for 5 min. The mixture was then subjected to 100°C for 5 min in order to stop the digestion. The electrophoretic pattern of the digested peptides was investigated by performing SDS-PAGE using 7.5% resolving gel. Peptide mapping of the collagen samples were repeated in the same protocol as described above without adding lysyl endopeptidase. The peptide map of PSC, type I calf skin collagen and type I salmon skin collagen were compared.

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrum of PSC was recorded from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1} using FTIR spectrophotometer (Agilent Cary 640; Agilent Technologies, Santa Clara, CA, U.S.A.). Attenuated Total Reflexion (ATR) mode was used in this investigation. The FTIR spectrum obtained was analyzed using Resolutions Pro FTIR software (Agilent Technologies, Santa Clara, CA, U.S.A.).

Determination of denaturation temperature

The denaturation temperature of PSC was determined from the intrinsic viscosity changes of the collagen. The investigation was performed according to the method described by Wang *et al.* (2008) with slight modification. A total of 20 ml of 0.03% (w/v) PSC in 0.1 M acetic acid was loaded into Ubbelohde viscometer and incubated at 10°C for 30 min. The temperature was increased from 10°C to 50°C stepwise at intervals of 2.5°C. The temperature was maintained at each designated temperature for 30 min. The efflux time of PSC solution was measured at each designated temperature. The efflux time of the blank solvent (0.1 M acetic acid) was also measured using the same protocol as described above. Fractional viscosity of PSC at a designated temperature was calculated according to the following equation:

$$\text{Fractional viscosity} = [\eta_{sp(t°C)} - \eta_{sp(50°C)}] / [\eta_{sp(10°C)} - \eta_{sp(50°C)}],$$

where the specific viscosity (η_{sp}) was calculated as follows:

$$\eta_{sp} = (t - t_0) / t_0,$$

where t is the efflux time of PSC and t_0 is the efflux time of the blank solvent. The thermal denaturation curve was obtained by plotting the fractional viscosity against temperature. The denaturation temperature (T_d) of PSC was determined as the temperature where the fractional viscosity was 0.5.

Results and Discussion

Extraction of PSC from the body wall of crown-of-thorns starfish (COTS)

The moisture content of the body wall of COTS was 79.43%. The yield of PSC was 2.29% (dry weight basis). After NaOH treatment, demineralization and disaggregation, the collagen of COTS was first extracted using 0.5M acetic acid but the collagen fibrils were not solubilized in 0.5M acetic acid. Therefore, no collagen can be collected at this step. However, at the subsequent step, collagen fibrils from COTS were easily solubilized by pepsin digestion. In addition, Saito *et al*. (2002) and Cui *et al*. (2007) also reported the similar results for collagen fibril from sea cucumber *Stichopus japonicas*. The insolubility of collagen fibrils in acetic acid indicates that the cross-links at the telopeptide region (non-helical region) of collagen molecules and the intermolecular cross-links of collagen molecule were not broken during the extraction with only acetic acid (Jongjareonrak *et al*., 2005). Therefore, these cross-links decreased the solubility of collagen in acetic acid (Jongjareonrak *et al.*, 2005). However, the collagen was easily solubilized by pepsin digestion. This is due to the cross-linkages at the telopeptide region were cleaved by pepsin without disrupting the triple helical structure of the collagen molecule (Jongjareonrak *et al*., 2005). Therefore, pepsin was used in the extraction of collagen from COTS.

Electrophoretic pattern of PSC

The electrophoretic pattern of the PSC from the body wall of COTS is shown in Figure 1. The SDS-PAGE shows that PSC consisted of two α chains, β (dimer) chain, γ (trimer) chain and high molecular weight cross-linked component (intra- and inter- molecular cross-link). The α chains were the major component of PSC. The result showed that α1 and α2 chains had different electrophoretic mobility, suggesting that their molecular weights were different. Furthermore, the β-component of PSC composed of β_{11} and β_{12} chains. The β_{11} is a homodimer of α1 chains whereas the β_{12} chain is the heterodimer of α1 and α2 chains. The SDS-PAGE pattern and subunit composition of PSC suggested that the PSC might be type I collagen which is consisted of two α1 chains and one α2 chain as the major component. In addition, the electrophoretic pattern of PSC was similar to those reported for collagen from purple sea urchin (Nagai and Suzuki, 2000), deep-sea redfish (Wang *et al*., 2008) and carp (Duan *et al.*, 2009). However, the electrophoretic mobility and molecular weight of the two α chains of PSC were significantly different from those of calf skin collagen and salmon skin collagens. The α1 and α2 chains of PSC appeared to have higher molecular weight compared to those of calf skin collagen and salmon skin collagen. Furthermore, the SDS-PAGE pattern of high molecular weight cross-linked component, γ and β chain of PSC were different from those of calf and salmon collagens. This suggested that the primary structure of PSC is different from those of calf skin collagen and salmon skin collagen. This discrepancy may also suggest that there is a difference between the primary structures of collagens from different animals.

Amino acid composition of PSC

Table 1 shows the amino acid composition of PSC. Glycine was the major amino acid found in PSC, accounting for 232 residues per 1000 residues of the total amino acid residues. This could due to the unique amino acid sequence of collagen where glycine appears at every third amino acid residues (Jongjareonrak *et al.*, 2005). However, the glycine content in PSC was lower than the theoretically expected glycine content, which was approximately 333 residues per 1000 residues. In addition, this result was in agreement with the glycine content of collagen from Nile perch (Muyonga *et al*., 2004) and Brownstripe red snapper (Jongjareonrak *et al*., 2005). Swatschek *et al.* (2002) reported that the low glycine content in collagen could be due to the impurities of glycoproteins which are tightly bound with collagen molecule. Furthermore, high content of hydroxproline (111 residues per 1000 residues), proline (108 residues per 1000 residues) and alanine (105 residues per 1000 residues) were detected in PSC. However, the amino acid analysis shows PSC has low content of phenylalanine (4 residues per 1000 residues) and tyrosine (7 residues per 1000 residues). Histidine was not detected in PSC. The relatively high content of glycine, proline and hydroxyproline, which are the characteristic amino acids of collagen, suggested that collagen was the major component of

Table 1. Amino acid composition of pepsin-solubilized collagen (PSC) from crown-of-thorns starfish (COTS)

Amino acids	Composition (residues/1000 residues)
Alanine (Ala)	105
Arginine (Arg)	94
Aspartic acid (Asp)	78
Glutamic acid (Glu)	106
Glycine (Gly)	232
Histidine (His)	ND[a]
Isoleucine (Ile)	19
Leucine (Leu)	16
Lysine (Lys)	18
Phenylalanine (Phe)	4
Proline (Pro)	108
Serine (Ser)	40
Threonine (Thr)	34
Tyrosine (Tyr)	7
Valine (Val)	28
Hydroxproline (Hyp)	111
Total	1000

[a]Not detected

PSC. In addition, the amino acid composition of PSC was similar to the amino acid composition reported for collagen from Nile perch (Muyonga *et al.*, 2004), Brownstripe red snapper (Jongjareonrak *et al.*, 2005) and deep-sea redfish (Wang *et al.*, 2008).

The total content of imino acid (proline and hydroxyproline) in PSC was 219 residues per 1000 residues, which was similar to that of collagen from calf and pig (215 and 220 residues per 1000 residues) (Zhang *et al.*, 2007). In addition, the imino acid content of PSC was found slightly higher compared to the collagen from tropical marine organisms (such as Nile perch and bamboo shark) which contain 180-207 residues of imino acid per 1000 of total amino acid residues (Muyonga *et al*., 2004; Kittiphattanabawon *et al*., 2010). However, it was much higher than the imino acid content of collagen from cold-water organisms (such as deep-sea red fish and cod) which contain 140-160 residues of imino acid per 1000 of total amino acid residues (Rigby, 1968; Wang *et al*., 2008). These observations suggested that the imino acid content in collagen of an organism is related to its living environment (Rigby, 1968). Furthermore, Jongjareonrak *et al.* (2005) showed that imino acid (proline and hydroxyproline) content contributed to the denaturation temperature of collagen. The pyrrolidine rings of proline and hydroxyproline stabilize of the secondary structure of polypeptide chains of collagen molecule. Therefore, this helps to maintain the integrity of the triple helical structure of collagen (Jongjareonrak *et al*., 2005). This also suggested that the stability of the structure of collagen was proportional to the total content of imino acid, that is, proline and hydroxyproline. In addition, the imino content of PSC was relatively high compared to collagen from other marine organism especially the cool-water marine organisms, suggesting that denaturation temperature of PSC was higher than other marine collagen.

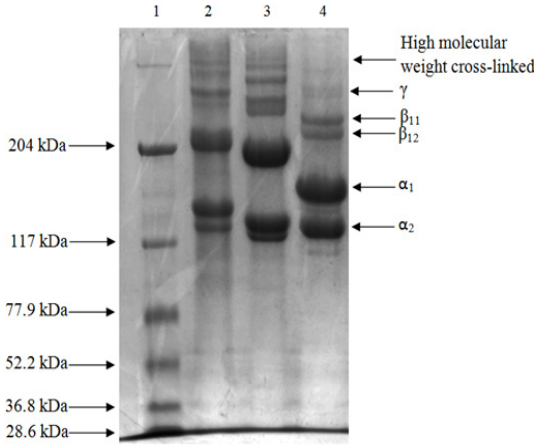

Figure 1. SDS-PAGE pattern of pepsin-solubilized collagen (PSC) from the body wall of crown-of-thorns starfish (COTS). Lane 1: broad range molecular weight marker; lane 2: type I collagen from calf skin; lane 3: type I collagen from salmon skin; lane 4: PSC

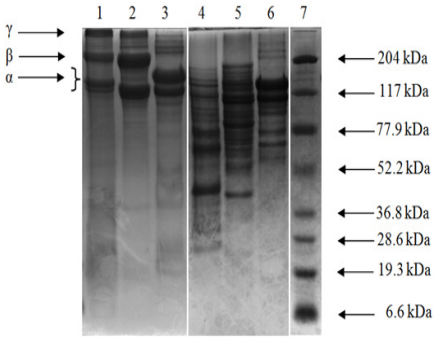

Figure 2. Peptide map of pepsin-solubilized collagen (PSC) from crown-of-thorns starfish (COTS) digested by lysyl endopeptidase. Lane 1: type I collagen from calf skin; lane 2: type I collagen from salmon skin; lane 3: PSC; lane 4-6: peptide fragments of digested type I calf skin collagen, type I collagen from salmon skin and PSC, respectively; Lane 7: broad range molecular weight marker

Peptide map of PSC

Peptide map of PSC digested by lysyl endopeptidase, in comparison with calf skin collagen and salmon skin collagen is shown in Figure 2. After the digestion by lysyl endopeptidase, the band intensities of high molecular weight cross-linked component, γ, β and α chains of PSC were decreased slightly compared to those of calf skin collagen and salmon skin collagen. This suggests that PSC was more resistant to lysyl endopeptidase digestion compared to the other two collagens. Furthermore, significant difference in the electrophoretic patterns of the digested peptides between PSC, calf skin collagen and salmon skin collagen was observed. This observation suggests that the availability of susceptible peptide bonds for lysyl endopeptidse digestion might be different in each collagen sample. Therefore, this contributed to different degree of

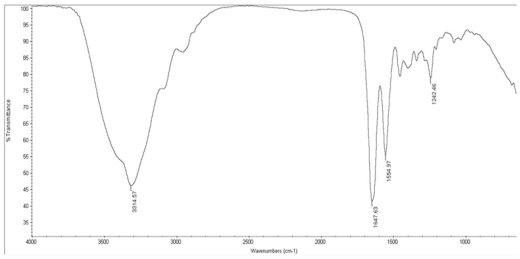

Figure 3. FTIR spectra of pepsin-solubilized collagen (PSC) from crown-of-thorns starfish and the positions of the major peaks in the spectrum

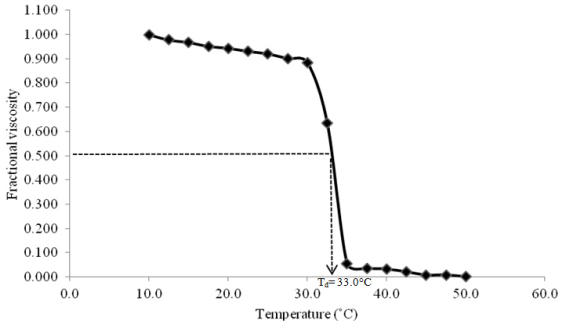

Figure 4. The thermal denaturation curves of pepsin-solubilized collagen (PSC). The denaturation temperature (T_d) of PSC was 33°C.

hydrolysis between the collagen samples. Lysyl endopeptidase is protease that cleaves peptide bonds at the carboxyl site of lysine residue. The content of lysine residue (18 residues per 1000 residues) (Table 1) in PSC was lower than those of calf skin collagen (26 residues per 1000 residues) (Zhang *et al*., 2007) and salmon skin collagen (26 residues per 1000 residues) (Saito *et al.*, 2001). This might contribute to the PSC being more resistant to the digestion by lysyl endopeptidase compared to calf skin collagen and salmon skin collagen. Therefore, the calf skin and salmon skin collagens were digested to a greater extent compared to PSC. There is a significant difference between the peptide maps of PSC, calf skin collagen and salmon skin collagen generated by lysyl endopeptidase. This suggested that the primary structure of PSC was different from calf skin collagen and salmon skin collagen.

Fourier transform infrared (FTIR) spectrum for PSC

FTIR spectrum of PSC and the positions of the major peaks are shown in Figure 3. The amide A is associated with N-H stretching frequency (Wang *et al*., 2008). The free N-H stretching vibration occurs in the range of 3400 - 3440 cm^{-1} (Wang *et al.*, 2008). However, when the NH group of a peptide involves in hydrogen bond, the position is shifted to lower frequencies (Duan *et al.*, 2009). The amide A band of PSC was located at 3314.57 cm^{-1}. This means that the NH groups of the collagen sample was involved in hydrogen bonding, which help to stabilize the triple helical structure of collagen. The amide I band of PSC was found at the wavenumbers of 1647.63 cm^{-1}. It was within the absorption range for amide I band, that is, 1600 - 1700 cm^{-1}. The amide I band was related to the stretching vibration of the carbonyl groups (C=O bond) along the polypeptide of collagen and it is also the indicator for peptide secondary structure (Wang *et al.*, 2008). The amide II band of PSC was appeared at 1554.97 cm^{-1}. It was fitting well in the characteristic absorption range for amide II band position (1550 - 1600 cm^{-1}) (Duan *et al.*, 2009). The amide III of the collagen sample was found at 1242.46 cm^{-1}, which was within the absorption range of amide III (1200 - 1400 cm^{-1}) (Woo *et al*., 2008). The amide III is related to NH bend and CH stretching (Woo *et al*., 2008). It is also associated with the triple helical structure of collagen (Woo *et al.*, 2008). Therefore, this suggested the triple helical structure of PSC was well preserved during the extraction. This result was similar to the FTIR spectra of collagens from deep-sea redfish (Wang *et al.*, 2008), carp (Duan *et al.*, 2009) and brownbanded bamboo shark (Kittiphattanabawon *et al*., 2010).

Thermal behavior

The hydrogen bonds that stabilize the triple helical structure of collagen were gradually broken as the temperature increase. This contributes to the disintegration of the triple helical structure into random coil structure through the thermal depolymerization (Wang *et al*., 2008). Therefore, a change in viscosity is observed (Wang *et al*., 2008). Figure 4 shows the change in fractional viscosity of PSC as the temperature is increased. The denaturation temperature of PSC was determined to be 33.0°C. It was 3.3°C and 4°C lower than the denaturation temperature of bovine collagen and porcine collagen, respectively (Ogawa *et al*., 2004; Zhang *et al.*, 2007). The denaturation temperature of collagen from COTS, which inhabit in tropical seawater, was similar to the collagen from other temperate and tropical marine organisms such as purple sea urchin (28°C), rhizostomous jellyfish (28.8°C) and grass carp (28.4°C) (Nagai and Suzuki, 2000; Nagai *et al*., 2000; Zhang *et al*., 2007). However, the denaturation temperature of PSC from COTS was much higher compared to the cold-water organisms such as deep-sea redfish (16.1°C), Alaska Pollack (16.8°C) and cod (15°C) (Wang *et al.*, 2008). These results suggest that the thermal stability of collagen is associated with the physiological and environmental temperature of the organism (Rigby, 1968). In addition, the denaturation temperature of PSC was higher than the denaturation temperature of collagen from other marine organism

may also due to its high imino acid content (219 residues per 1000 residues) (Muyonga *et al*., 2004). The structure of collagen is mainly stabilized by the pyrrolidine rings of proline and hydroxyproline which maintain the secondary structure of the polypeptide chain (Jongjareonrak *et al*., 2005). Furthermore, the hydrogen bonding of the hydroxyl group of hydroxyproline also helps to stabilize the structure of collagen (Zhang *et al*., 2007).

Conclusion

There were huge amount of crown-of-thorns starfish (COTS) were removed from the sea and treated as waste. In this study, collagen was successfully extracted from the starfish. The denaturation temperature of the collagen from COTS was comparable to bovine and porcine collagen. This suggested that COTS could be the potential alternative source of collagen. Furthermore, this is an environmental friendly approach to manage the underutilized starfish.

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