Collagen from Sea Cucumber (Stichopus variegatus) as an Alternative Source of Halal Collagen

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Abstract—Collagen is a kind of proteins which becomes the main component of teeth, muscles, flesh, bone, and skin layer. It is generally used in various fields such as food, cosmetics, pharmaceutical and medical. Collagen which was commercialized in the market is derived from skin and bones of land-based mammalian such as calf, pig, and sheep. While it was being reported that there were many diseases spread by land-based mammalian in the last few years such as mad cow disease and avian flu. Besides that, pigs is forbidden to be consumed by moslem since Islam teaches them to consume halal food. Marine organism has a potential to be used as an alternative source of halal collagen. One of them is sea cucumber. Sea cucumbers, especially *Stichopus sp,* has a potential to be used as an alternative source of halal collagen instead of mammals. Nevertheless, the extraction of sea cucumber collagen still relies on pepsin enzyme from pig derivatives. The exploration of source and extraction process is urgently needed to gain halal collagen. Therefore, the aim of this study was to explore sea cucumber *Stichopus variegatus* as a source of halal collagen with acid extraction method. Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries Jakarta (*sponsors).* This study was divided into two steps: extraction and characterization of collagen. Extraction of collagen produced yield of 16.40%. Characterization of collagen showed that the major components of amino acid were glycine (169 residues/1000residues), glutamic acid (111residues/1000 residues), proline (67 residues/1000 residues), alanine (64 residues/1000 residues) and molecular weight of collagen was 130.33 kDa. Collagen from sea cucumber *Stichopus variegatus* had similar characteristics to commercial collagen even though the yield of extraction was lower. This kind of collagen can be used as an alternative source of halal collagen either based on the source or the extraction process.

Keywords—*collagen, halal, sea cucumber.*

INTRODUCTION

Collagen is a kind of connective tissue protein in the form of fiber which constructs one third parts of the vertebrate body and becomes the main component of teeth, muscle, bone, and skin layer. Collagen has an unique structure for triple helix-shaped primary structure which means 3 polypeptide chains spiral Gly-X-Y where X and Y are usually composed of proline or alanine [1]. Collagen also has an unusual amino acid, namely hydroxyproline and hydroxylysine, both formed by the hydroxylation process. Collagen divided into 28 types, but 90% of them are I, II, III, and IV types [2].

Collagen is widely used in various fields such as food, cosmetics, pharmaceutical and medical. The nature characteristics of collagen are biodegradable, biocompatible and high antigenicity so that collagen is often chosen as raw material [3]. Collagen that had been commercializing in the market is collagen derived from skin and bones of land-based mammalian such as pig skin (46%), beef bones (23%), and chicken. While it was being reported that there were many diseases spread by land-based mammalian in the last few years such as mad cow disease and avian flu [4].

Islam teaches moslem to consume halal food which is good for man health as Allah said in QS. Al-Baqarah 168-and 173. Awareness of the importance of halal and healthy food in society is increasing in the last few decade. It is proved by the attempts of people to find the alternative sources of halal collagen. Accordingly, it is necessary to explore source of collagen which more safety instead of collagen from pis, chicken, and cow. Marine organism has a great potential to be developed as a source of halal collagen, such as fish, shellfish, and sea cucumbers.

Sea cucumber is one of echinoderm (thorn-skined animals), but not all of it has thorns. The thorn is actually a skeleton which is composed of lime embedded in the skin [5]. Sea cucumber has spherical and cylindrical shape with body length about 10-30 cm. Sea cucumber has vary color from dark, brown, gray, red, dark green and orange. It movement was very slow. It is found in the bottom of the sea with a depth of 1-40 m [6]. There are about 23 species that have been identified in Indonesia [7]. Sea cucumbers wich has highest economic value are from genus Holothuria, Muelleria, and Stichopus. Species from genus of Stichopus such as *Stichopus vastus, Stichopus cloronotus, Stichopus quadrifascinatus,* and *Stichopus variegatus* [8].

Recent researches reported that sea cucumber has a great potential to be used as a source of collagen, including *Stichopus japonicus* [9], *Stichopus vastus* [10], *Stichopus monotuberculatos* [11], and *Bohadschia Bivitatta* [12]. Collagen from sea cucumber has physicochemical characteristics that resemble to commercial collagen. In general, collagen which is extracted from sea cucumber uses PSC (*pepsin solubilized collagen*) method. This method needs pepsin enzyme which is obtained from porcine (pig). The using of pepsin enzyme on collagen extraction makes the gained collagen not halal although it has been derived from halal sources. There are 3 kinds of collagen extraction methods, namely acidic, basic, and enzymatic. The exact method of collagen extraction from sea cucumber needs to be done so that the product can be stated completely halal, either based on the source or the extraction method. Therefore, the purpose of this study was to explore the potential of sea cucumber *Stichopus variegatus* collagen by using ASC (*acid solubilized collagen*) method as a source of halal collagen.

MATERIALS and METHODS

Sea cucumbers *Stichopus variegatus* (average body weight 300 g) were purchased from local fisherman in Lampung Province, Indonesia. The fresh body wall of *S. variegatus* were dissected free of adherent tissues with tweezer, cut into small pieces and stored in polybag at -20 ^{0}C until used. The collagen were extracted from body wall of *S. variegatus* according to the procedures described by Park *et al* [9] with some modifications. All procedures were performed at 4 ^{0}C. A hundred gram of sea cucumbers were homogenized with 1000 ml of distilled water and stirred slowly for 30 minutes. The water was replaced and the extraction in water was repeated once for 1 hour. Then the water was replaced with 200 ml of 50% ethanol and stirred slowly for 30 minutes. Samples were replaced with distilled water and then incubated with 1000 ml of a disaggregating solution containing 0.1 M Tris-HCl pH 8.0, and 4 mM ethylenediaminetetraacetic acid (EDTA) overnight. The liquid was decanted and replaced with 1000 ml of distilled water, in which the samples were stirred slowly for 30 minutes.

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The disaggregated collagen fibrils was collected and treated with 1000 ml of 0.1 M NaOH for 48 hour in order to remove non-collagenous materials effectively and to minimize the effect of endogenous proteinases on collagen fibrils. The residue after alkali extraction was thoroughly rinsed with distilled water and then soaked in 1000 ml of 0.5 M acetic acid for 48 hour. The suspension was filtered through cheesecloth and then filtrate was precipitated using 1 M of NaCl overnight. The filtrate was centrifuged at 10.000*g* for 60 minutes and then pellet was dissolved in 0.5 M acetic acid. It was then dialyzed in 0.1 M acetate buffer overnight. Buffers should be periodically replaced. The pellet was finally lyophilized using a freeze dryer (Christ alpha 1-2 LD, USA). Amino acid of collagen which was extracted from *S. variegatus* was characterized by using HPLC instrument (*High Performance Liquid Chromatography*), while its molecular weight was characterized by using SDS-PAGE instruments *(Sodium Deodecyl Sulphate Poly-Acrilamide Gel Electrophoresis)*.

Table 1. Amino Acid Composition of Collagen from Sea Cucumber *Stichopus variegatus* Compared With Sea Cucumber *Stichopus japonicus,* Calf, and Pig Skin Collagen (Residues/1000Residues)

Amino acid	Sea cucumber collagen[1]	Sea cucumber collagen[2]	Calf skin collagen[3]	Pig skin collagen[4]
Asp	58	59	45	44
Thr	37	33	18	16
Ser	26	44	33	33
Glu	111	109	75	72
Pro	67	95	121	123
Gly	169	325	330	341
Ala	64	112	119	115
Cys	0	0	0	0
Val	18	23	21	22
Met	8	9	6	6
Ile	15	21	11	10
Leu	23	18	23	22
Tyr	12	5	3	1
Phe	13	3	3	12
Lys	10	7	26	27
His	5	4	5	5
Arg	66	55	50	48

Source: [1](This research), [2](Saito *et al.* 2002), [3](Giraud *et al.* 2000), [4](Ikoma *et al.* 2003).

Fig. 2 shows SDS-PAGE pattern of collagen from the body wall of sea cucumber *S. variegatus* that has electrophoretic pattern of type I collagen consisting of major component α_1 of approximately 130.33 kDa. Based on its molecular weight, collagen from *S. variegatus* showed a similar molecular weight to α_1 chain of collagen from *S. monotuberculatos* (137 kDa [11]), *S. japonicus* (135 kDa [13]), *P. californicus* (138 kDa [17]), and *Bohadschia bivitatta* (138 kDa [12]). Previous result suggested that the collagen from sea cucumber is type I, consisting of an $(\alpha)_2\beta$ heterotrimer or an (α) homotrimer [16]. The addition of mercaptoethanol did not affect the SDS-PAGE pattern, suggesting that there was no molecular species with disulphide bonds. Type I collagen usually consists of heterologous α_1 and α_2 chains forming triple helix as $(\alpha_1)_2\alpha_2$, where β and γ component of higher molecular weight are dimer and trimer of α chain, respectively [13]. Liu *et al* [20] pointed that collagen from chicken feet consisted of two type collagen, one is type I and another is type II, but the major component is type I. All collagen types consisted of three polypeptide chain (α-chains) in close association.

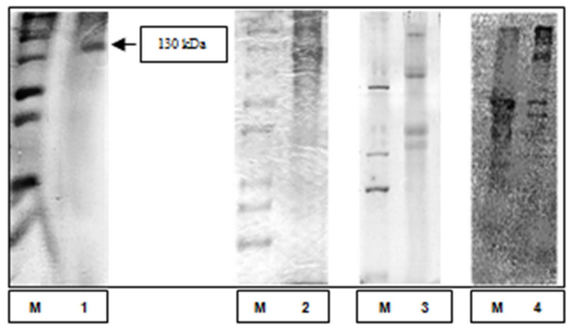

Figure 2: SDS-PAGE of collagen from 1. sea cucumber Stichopus variegatus (this research); 2. sea cucumber Bohadschia bivitatta (Siddiqui et al. 2013); 3. calf skin (Zhong et al. 2015); 4. Chicken feet (Liu et al. 2001); M = marker.

DISCUSSION

Collagen and its derivatives are currently the intermediate materials which are widely used in various industries both medium and large scale. Collagen demand of the world continues to grow year by year. Collagen is most widely used in cosmetic and medical fields in Indonesia. However, the supply of collagen Indonesia still depends on import nowadays. Domestic production can not compete with the global market since the amount of production is not sufficient to fulfill the demand collagen. Besides, quality of collagen which was extracted in our country is still lower than international standards.

The main source of collagen which is usually used are from land-based mammalian such as calf, pig, and sheep. During the past 10 years, many emerging issue of animal diseases happened in Asian sountry, such as madcow disease and avian flu which attacks cattle and poultry. Even, collagen from cow and chicken is plentifully produced in Indonesia. The number of outstanding collagen from pig is also increase gradually. That is why the issue of halal source of collagen becomes urgent in society.

Islam teaches Moslem to be careful in choosing food and beverages. Islam recommends to consume food which is lawful and good for health. It is stated in QS. Al-Baqarah 168 and 173. It is also re-emphasized in QS. An-Nahl 115. Halal means the souce and manufacturing process in making food and beverages are appropriate with what Islam was taught about. Food which was derived from unhealthy source is harmful for man health. A lot of reports about disease in land-based mammalian the past decade encourages moslem to find halal sources of collagen.

Indonesia has vast areas of oceans beyond the mainland. Based on that fact, the potential of marine resources should be able to meet the needs of collagen over the country. The exploration of marine resources as a source of collagen has been actually researched and developed as from squid, skin, bones, and swim bladder of fish. Beside those sources, sea cucumber also has great potential to be developed since the production of sea cucumber in Indonesia per year reach about 184 thousand tons. Sea cucumber is commonly exported in fresh and dried form. The resale value of sea cucumber will may be increase only if sea cucumber is processed into intermediate materials such as collagen.

Stichopus variegatus is potentially to be developed as a source of collagen based on this research result. Research in Europe and Japan has been widely reported that *Stichopus sp* has a high content of collagen. It is also confirmed by this research. Mass production and ongoing exploration will be able to meet the needs of collagen in Indonesia so that collagen produced in this country can compete with imported collagen. Yet, it takes great effort and cooperation of all parties to reach it.

CONCLUSION

Collagen of *Stichopus variegatus* had a yield of 16.40 % dry basis with the primary amino acid composition in the form of glycine, proline, glutamic acid, and alanine with a molecular weight of 130.33 kDa. It had similar chemical characteristics to cow, pig, and chicken collagen. It can be used as an alternative source of halal collagen. Researchers suggest that physical properties of collagen from sea cucumbers should be tested and compared with commercial collagen. In addition, the extraction methods of collagen need to be reviewed in order to gain high yield and good quality

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