Sea Cucumber Viscera Hydrolysate as a Potential Animal Feed Supplement

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Abstract. Among the seafood items, Sea Cucumbers are one of those popular seafood products in the Chinese community, fetching with reasonable high pricing. A major issue with Sea Cucumber is the waste generated up to 30-40% waste. Sea Cucumber internal Organs (SCiO) and washed water, containing soluble components usually discarded as waste in the industry has considerable amount of nutritional components. A preliminary study by Universiti Kebangsaan Malaysia (UKM) research team recently discovered that, on dry weight basis, the SCiO contains 50.27% and 22.16% of protein and fat contents respectively. Amino Acids Profile (AAP), Fatty Acids Profile (FAP), soluble minerals and peptides of the various components obtained from the processing steps, i.e., hydrolysis, filtration, membrane separation, concentration and finally spray drying and/or freeze-drying of the SCiO were determined. Overall, SCiO contains all the 9 essential amino acids, which corresponds to 36.65 ± 0.06 % of the total amino acids. Aside from that, the SCiO oil contains 39.76 ± 0.29 % saturated fatty acids, 26.86 ± 0.01 % monounsaturated fatty acids, and 33.39 ± 0.28 % polyunsaturated fatty acids. Interestingly, the Omega-3 eicosapentaenoic acid (EPA) (C20:5n3), an essential fatty acid, was found to be the major fatty acid of SCiO hydrolysates, amounting to 27.60 ± 0.14 % of total oil content. This corresponds to approximately 6.5-7.2% of the SCiO hydrolysates being EPA, which is high and valuable, especially coming from a waste material. Thus, there is a huge potential to recover these functional components, together with the other potential functional properties, from the SCiO hydrolysate that may contribute as animal nutritional feed supplement.

1. Introduction
Malaysia is well known in Asia Pacific as a producer of premium value-added seafood products, such as Abalones, Sea Cucumbers and Lobsters, for domestic and overseas market; exporting to East Asia, Australia, New Zealand, North America and Europe. In recent years, due to high demand of such seafood products worldwide, local resources have become scarce, and many of these items had to be out source from other countries in the Asia Pacific, etc. Among the seafood items, Sea Cucumbers are one of those popular seafood products in the Chinese community, fetching with reasonable high pricing. However, a major issue with Sea Cucumber is the waste generated in the process, resulting up
to 30-40% waste in the form of visceral organs, which, if the waste not managed properly, it might pose as environmental issue to the community, such as causing pollution and contamination.

Carbohydrates, fat and proteins are the macronutrients that needed in quite large amounts upon consumption. Minerals and vitamins, the micronutrients, are necessary for physiological and biochemical processes in the human body to maintain proper health [1]. Sea Cucumber internal Organs (SCiO) and washed water, containing soluble components usually discarded as waste in the industry has considerable amount of nutritional components. A preliminary study by Universiti Kebangsaan Malaysia (UKM) research team recently discovered that, on dry weight basis, the SCiO contains 50.27% and 22.16% of protein and fat contents respectively.

In order to evaluate the potential benefits on the nutritional quality of the SCiO, researchers from UKM in collaboration with Agroidon Technologies have developed an efficient biotechnology process of using selective enzymes to hydrolyse the internal organs, converting insoluble visceral organs and connective tissues into highly functional and nutritious components, using optimum conditions to obtain high yield of SCiO. Subsequently Amino Acids Profile (AAP), Fatty Acids Profile (FAP), microbiology quality, contents of minerals, vitamins, heavy metals and peptides of the various components after the processing steps, i.e., hydrolysis, filtration, membrane separation, concentration and finally spray drying and/or freeze drying of the SCiO were determined.

2. Materials and methods

2.1. Materials
A sea cucumber internal organ (SCiO) was provided by Harmony Marine Products Sdn. Bhd., Pasir Gudang, Johor, Malaysia. Two enzymes were used, i.e. Alkaline Protease ST and Exopeptidase ST purchased from Science Technics Sdn Bhd, Klang, Selangor, Malaysia.

2.2. Enzymatic hydrolysis of SCiO
SCiO was cut into smaller pieces to increase the surface area for the enzymatic hydrolysis process. The SCiO was adjusted to approximately 20% total solids with water, and pH adjusted to pH 8 with sodium hydroxide. Temperature was set at 60°C, added 1% of the Alkaline Protease ST enzyme, and held for 3 hours with stirring. Subsequently, 0.5% of Exopeptidase ST enzyme was added and kept at 60°C for 18 hrs with stirring. The insoluble fraction was filtered off, and the filtrate was refrigerated at 4°C to allow fat separation. The separated fat (on the surface) was scooped out from the samples, to obtain the SCiO oil. The liquid was spray dried to produce SCiO hydrolysates in powder form.

2.3. Physicochemical Characterisation of SCiO Hydrolysates
The solubility (total soluble solids), water activity, and the proximate compositions of SCiO Hydrolysates were determined [2].

2.4. Estimation of mineral, vitamin and heavy metal contents
Mineral, vitamin and heavy metal contents of SCiO hydrolysates were quantified using atomic absorption spectrometry (AAS), high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP/MS), respectively, after microwave digestion process.

2.5. Amino acid profiling
Amino acid profiling of SCiO hydrolysates was conducted using 3 different hydrolysis methods (acid, performic acid, and alkaline), and subsequently injected into HPLC for separation. Appropriate standards were used to identify and quantify the amino acids present [3].
2.6. Fatty acid profiling
Fatty acid profile of SCiO oil was determined using the fatty acid methyl ester (FAME) method, coupled with GC-FID [4]. The fatty acid present, together with its amount were identified using appropriate standards.

2.7. Microbiology profiling
Microbiology profile of SCiO hydrolysates was performed according to method of Food and Drug Administration (FDA): Bacteriological Analytical Manual Online 2012, 2013, 2016 and method ISO 6779-1: 2017. The microbiological analyses performed were total plate count, coliform, E. coli, S. aureus, Salmonella spp., and yeast and mould.

3. Results and discussion

3.1. Recovery of SCiO hydrolysates
On dry weight basis, approximately 6 kg of SCiO was utilised for the enzymatic hydrolysis, of which 5.83 kg of spray dried SCiO hydrolysates powder was produced. This translates to 97% recovery of the SCiO hydrolysate. Simultaneously, 140 g of SCiO oil was produced, which translates to approximately 2.4% recovery of the oil. Overall, these figures were very convincing, and thus, translating to a very sustainable model, in which negligible waste was produced.

3.2 Physicochemical xharacteristics of SCiO hydrolysates
The major composition of SCiO hydrolysates was protein and fat, at 49.4% and 24.2% respectively, as shown in Figure 1. This is consistent with the preliminary work performed, where the raw SCiO contains 50.27% and 22.16% of protein and fat respectively. This shows that the enzymatic hydrolysis did not alter the nutritional properties of the SCiO.

![Proximate Composition of SCiO Hydrolysate](image)

**Figure 1.** Proximate composition of SCiO hydrolysates

Aside from that, Table 1 show that the water activity of the SCiO hydrolysates were very low, at 0.35±0.004, compared to that of the raw SCiO, at 0.57±0.001. The decrease in the water activity is due to the spray drying process, which produces a powder with very high stability. The total solubility of
the SCiO hydrolysates also increased to 66.4%, from 42.9% of the raw SCiO. The increase in solubility was attributed to the enzymatic hydrolysis performed, where proteins were hydrolysed into peptides and amino acids, which in turn, are more soluble in water.

| Table 1. Water activity and total solubility of the raw SCiO and SCiO hydrolysates |
|------------------|------------------|
| Samples          | Water activity ($A_w$) | Total solubility |
| Raw SCiO         | 0.57 ± 0.001      | 42.9%           |
| SCiO hydrolysate | 0.35 ± 0.004      | 66.4%           |

3.3. Mineral contents

Sea Cucumber internal Organs (SCiO) have very high content of essential minerals as shown in Table 2. Calcium, iron and sodium were high in SCiO raw materials. The potassium content was low whereas magnesium was not detected in the SCiO raw internal organs. In SCiO hydrolysate, most of the minerals were reduced whereas the magnesium was found in this sample. This magnesium exists in a very low amount within the raw SCiO, a trace amount that could not be detected compared to SCiO hydrolysate sample after hydrolysis process and converted into powder form. Thus, it can be useful in animal feed formulation and even as health supplement for Human being.

| Table 2. Minerals content of SCiO raw materials and SCiO hydrolysate |
|------------------|------------------|------------------|
| Parameter        | SCiO raw (g/100g) | SCiO hydrolysate (mg/kg) | Recommended nutritional intake (Ministry of Health, Malaysia) |
| Calcium          | 128.3            | 2617.2            | 1000-1200 mg/day |
| Iron             | 39.7             | 820.5             | 14 mg/day (10%) |
| Potassium        | 0.05             | 4602.9            | 4.7 g/day |
| Magnesium        | -                | 3340              | 310-420 mg/day |
| Sodium           | 338.3            | 17447.7           | 1500 mg/day |

3.4. Vitamins content

SCiO hydrolysates contain high vitamins concentration, as shown in Table 3. Intake of relatively small quantities of SCiO hydrolysate may cover the requirements for some vitamins in animal as well as human nutrition [5]. SCiO hydrolysate can thus be considered to represent a potentially good source of vitamins or as a vitamin supplement for animal and/or human nutritional benefits.

| Table 3. Vitamins content of SCiO hydrolysate |
|------------------|------------------|
| Parameter, unit  | SCiO hydrolysate | Recommended nutritional value/ daily (RNVD) – Food Regulation (1985) |
| Vitamin B1 (Thiamine), mg/100g | 8 | 2.2 mg |
| Vitamin B2 (Riboflavin), mg/100g | 45.8 | 3.2 mg |
| Vitamin B3 (Nicotinic acid), mg/100g | 94.7 | 22 mg |
| Vitamin B6 (Pyridoxine), mg/100g | 6 | 4.0 mg |
| Vitamin B5 (Pantothenic acid), mg/100g | 17 | 14.0 mg |
| Vitamin B7 (Biotin), mg/kg | 0.2 | 400 µg |
| Vitamin B9 (Folic acid), ug/kg | 107 | 400 µg |
| Vitamin B12 (Cobalamin), ug/kg | 1222 | 4 µg |
| Vitamin C, mg/100g | 5.5 | 100 mg |
| Vitamin E as a-tocopherol, mg/100g | 0.14 | 50 I.U. |
Vitamin A as β-carotene, mg/100g  2.3  5,000 I.U.
Vitamin D, μg/kg  37.5  800 I.U.
Vitamin K, mg/kg  1.2

3.5 Heavy metals content
Based on Food Regulations 1985 (14th Schedule), the amount of arsenic and cadmium exceeded the permissible amount after hydrolysis process. This data showed that the arsenic and cadmium were accumulated in the SCiO hydrolysate powder, as shown in Table 4, and can be harmful to health. Most of the living organisms need small amount of essential metals such as Fe, Mn, Cu, and Zn for essential processes such as growth [6]. However, all these metals will give harmful effects when exceeding the standard limits. The heavy metals such as Cd, Pb, As, and Hg are known as nonessential metals. They are toxic even in small amount and harmful to human health [7]. However, since SCiO is deemed suitable as a potential ingredient for animal feed formulation and Health supplements (due to the rich nutrients of protein, lipid and other essential components, the high contents of heavy metals can be formulated to meet the maximum permissible limit, based the feed act & regulation. If necessary, additional processing technology such as ion-exchange chromatography can be utilized to reduce/remove these heavy metals in the SCiO hydrolysates.

| Parameter (mg/kg) | SCiO raw | SCiO hydrolysate | Food Regulation (1985) |
|------------------|----------|-----------------|------------------------|
| Arsenic          | 1.57     | 6.93            | ≤ 1                    |
| Mercury          | -        | 0.004           | ≤ 0.5                  |
| Cadmium          | 0.43     | 1.71            | ≤ 1                    |
| Lead             | 0.89     | 0.58            | ≤ 2                    |
| Antimony         | 0.02     | 0.003           | ≤ 1                    |
| Manganese        | -        | 16.2            | -                      |
| Phosphorus       | -        | 2857.1          | -                      |
| Selenium         | -        | 3.54            | -                      |
| Stanum           | 0.33     | -               | ≤ 40                   |

3.6 Amino acid profile
Overall, SCiO hydrolysates contain all the 9 essential amino acids, which correspond to 36.65 ± 0.06 % of the total amino acids present, as shown in Table 5. Among the major amino acids were glutamic acid (7.36 ± 0.02 %), aspartic acid (5.64 ± 0.03 %), arginine (5.24 ± 0.02 %), leusine (4.24 ± 0.01 %) and threonine (4.19 ± 0.04 %). Based on recent studies reported on visceral hydrolysates of Abalone and sea cucumber, the peptides and glycopeptides exhibited high antioxidant activities and showed potential functional role in reducing hypertension [8, 9, 10, 11].
Table 5. Amino acid profile of SCiO hydrolysates (n=2)

| Amino acids   | Amount (g/100g) |
|--------------|-----------------|
| Hydroxyproline | 0.21±0.01       |
| aspartic acid  | 5.64±0.03       |
| serine        | 3.59±0.02       |
| glutamic acid | 7.36±0.02       |
| glycine       | 4.22±0.03       |
| histidine*    | 1.24±0.02       |
| arginine      | 5.24±0.02       |
| threonine*    | 4.19±0.04       |
| alanine       | 2.18±0.03       |
| proline       | 2.75±0.01       |
| thyrosine     | 2.06±0.02       |
| valine*       | 2.36±0.01       |
| methionine*   | 1.08±0.02       |
| lysine*       | 1.11±0.01       |
| isoleusine*   | 2.15±0.01       |
| leusine*      | 4.24±0.01       |
| phenylalanine*| 2.23±0.01       |
| tryptophan*   | 0.65±0.01       |
| cysteine      | 0.01±0.01       |
| **Total**     | **52.52±0.25**  |

*essential amino acids

3.7. Fatty acid profile

SCiO oil contains 39.76 ± 0.29 % saturated fatty acids, 26.86 ± 0.01 % monounsaturated fatty acids, and 33.39 ± 0.28 % polyunsaturated fatty acids, as shown in Table 6. Interestingly, the Omega-3 eicosapentaenoic acid (EPA) (C20:5n3), an essential fatty acid, was found to be the major fatty acid of SCiO hydrolysates, amounting to 27.60 ± 0.14 % of total oil content. This corresponds to approximately 6.5-7.2% of the SCiO hydrolysates being EPA, which is high and valuable, especially coming from a waste material. Among others, EPA, together with docosahexaenoic acid (DHA), has been associated with foetal development, cardiovascular function, and Alzheimer's disease [12].

Table 6. Fatty acids of SCiO oil (n=2)

| Fatty acids   | Saturated fatty acids | Monounsaturated fatty acids | Polyunsaturated fatty acids |
|---------------|-----------------------|-----------------------------|----------------------------|
| SCiO Oil      | 39.76 ± 0.29 %        | 26.86 ± 0.01 %              | 33.39 ± 0.28 %             |

3.8 Microbiology profile

The microbiological quality of the SCiO hydrolysates is shown in Table 7. The results were compared with the microbiological guidelines or standards imposed by Ministry of Health (MOH) Malaysia and the other international standards. The total plate count, coliform, *E. coli*, *S. aureus*, *Salmonella* spp., yeast and mould counts of the SCiO hydrolysates were within the acceptable limit [13, 14, 15]. The microbial load and the presence of pathogenic microorganisms in food will reflect the food hygienic quality and the associated potential health hazards [16]. Thus, the microbial counts within the acceptable limit are important to make sure the food products are safe for consumption.
Table 7. Microbiology counts of SCiO hydrolysate (n=2)

| Colony count                  | Amount        |
|-------------------------------|---------------|
| Total plate count (CFU/g)     | $3.1 \times 10^2$ |
| Coliform (MPN/g)              | < 3           |
| E. coli (MPN/g)               | < 3           |
| S. aureus (MPN/g)             | < 3           |
| Salmonella spp. in 25 g       | Absent        |
| Yeast and mould (CFU/g)       | $1.5 \times 10^2$ |

CFU = colony forming unit
MPN = most probable number

4. Conclusion
Enzymatic hydrolysis process has successfully converted the SCiO waste material into potential products for animal nutrition feed supplement and health supplements agro-based food and health industries. Two major components were derived from the SCiO, i.e. the SCiO hydrolysates and the SCiO oil. The application of these functional products as animal nutritional feed supplement, functional food ingredients, nutraceutical and pharmaceutical applications are being studied. This research showed the potential processing technology of being able to convert the waste material into high-value products, which in turn, enhances the economic value of SCiO, and help reduced the waste that can lead to unhealthy environmental contamination.

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