

## Original Research Article

# **Holothuria atra: An Underutilized Marine Resource for Nutritional and Collagen Benefits**

### ABSTRACT

**Aims:** To assess the nutritional content in the body wall of an underutilized sea cucumber species: *Holothuria atra* and to extract and characterize the potential collagen types from the body wall. **Study design:** These specimens of sea cucumber were identified based on morphology and then nutritionally analyzed with respect to lipid profile, vitamins, minerals, and carbohydrates. *H. atra* body wall was used to extract collagen and later characterized.

**Place and duration of study:** Samples were collected at Mannar, Sri Lanka. Study was conducted at Department of Zoology, University of Sri Jayewardenepura, Sri Lanka during 1 year. **Methodology:** Nutritional content in the body wall (proximate analysis, lipid profile, vitamins, minerals, and carbohydrates) was analyzed using biochemical assays. Moreover, *H. atra* body wall was used to extract collagen, by acid-soluble collagen extraction and characterized by physico-chemical methods.

#### **Results:**

The results showed that body wall tissues of *H. atra* contained high moisture level (83.2%), proteins (10.2%) and low levels of fat (2.0%) and carbohydrates (2.1%). Further, flesh contains, 0.4% saturated fatty acids, 0.4% unsaturated fatty acids, considerable amount of Calcium, Magnesium and Sodium. Collagen yield from the body wall was recorded as 0.95% and fibrils observed as irregular and dense with loose and porous structure. Attenuate Total Reflectance-Fourier Transform Infrared Spectroscopy and Ultra Violet Vis spectroscopy indicated the collagen was Type I.

**Conclusion:** Results suggest that underutilized, non-commercial *Holothuria atra* is a potential nutritional source and it contains type I collagen. Further studies are underway to increase the collagen yield from *H. atra* and to develop a collagen membrane which can be used in future industrial applications.

Keywords: *Holothuria atra*, marine collagen, marine genetic resources

## 1. INTRODUCTION

*Holothuria atra* (Lollyfish) is a non-commercial sea cucumber species available in Sri Lanka coast, especially along the North-West region [1]. It has long been consumed in many regions of the world as a potential biological source against aging, oxidation, inflammation, microbial activity, wound setc [2]. *H. atra* flesh is high in protein and further, it contains significant levels of essential and non-essential amino acids [3]. Sea cucumbers are known to offer remarkable nutritional profile and have

8 attracted the attention in both culinary and health perspective. Therefore, it is worthwhile to investigate  
9 full nutritional profile of non-commercial, underutilized sea cucumber species, such as *H. atra*. Recent  
10 research related to isolating bioactive compounds such as collagen, flavonoids, phenolic components,  
11 terpenoids, saponins, alkaloids, acid mucopolysaccharide and triterpene glycoside from *H. atra* has  
12 shown sufficient evidence for unique industrial values of this species [2].

13 Being a promising option among “blue materials” which can substitute mammal-derived collagen,  
14 marine collagen has many applications in the fields of medicine, cosmetics, pharmaceuticals and food  
15 industries [4]. Further, they are rich in antioxidant properties, environmentally friendly extraction  
16 procedures, low molecular weight, minor regulatory and quality control problems, a negligible number  
17 of biological contaminants and toxins, low inflammatory response and excellent metabolic  
18 compatibility, free of infectious diseases, free of allergies and avoiding religious concerns [4]. Due to  
19 its unique structural properties, marine collagen is being used in food industry as additives, packaging  
20 material, dietary supplement, functional food, confectionery and desserts while used as a  
21 multifunctional biomaterial in tissue regeneration, wound dressing, etc [5]. Bioactive properties such  
22 as anti-aging and anti-wrinkling activities enable the use of collagen to formulate lotions and gels with  
23 high moisturizing action with UV protective properties that are important in cosmetic industry [5].  
24 Sea cucumber-derived collagen fibrils frequently show symmetrical spindle shape and short length [6].  
25 They are bipolar molecules with a surface associated with proteoglycans [6]. Covalent internal  
26 crosslinks are very similar to mammalian collagen yet permanent crosslinks are absent in the  
27 structure which facilitates isolation of collagen, avoiding mechanical damage. Moreover, absence of  
28 permanent crosslinks help to slide past one another in shortening and lengthening process among  
29 fibrils [6]. Type I collagen is the most common and abundant collagen type in marine invertebrates

1 with two equivalent  $\alpha 1$  and one  $\alpha 2$  polypeptide chains which composed of  $1.1 \times 300$  nm size collagen  
2 molecules [7].

3 Full nutritional profile of sea cucumbers can be investigated using well established protocols.

4 Collagen which is a potential protein of sea cucumbers can be extracted using conventional and  
5 novel methods. Characterization of extracted collagen is essential prior to investigating on commercial  
6 applications. However, present work aimed to analyse the full nutritional profile of *H. atra*,  
7 underutilized sea cucumber species in Sri Lanka and obtain collagen to examine their  
8 physicochemical characteristics and structure. Investigations on collagen extracted from these sea  
9 cucumber are scanty, compared to other marine species. There is growing interest on the non-  
10 commercial marine species with respect to bioprospecting. Accordingly, the present study provides  
11 the basis for nutritional quality, extraction and characterization of collagen from *Holothuria atra*.

## 12 **2. MATERIALS AND METHODS**

### 13 **2.1 Sample collection**

14 Live specimen of *H. atra* were obtained commercially from Mannar, Sri Lanka and stored in ice. The  
15 viscera of sea cucumber samples were immediately removed and the body wall was diced and kept  
16 at  $-20^{\circ}\text{C}$  until further use.

### 17 **2.2 Nutritional composition analysis of *Holothuria atra***

#### 18 **2.2.1 Proximate composition of *Holothuria atra***

19 The diced tissue samples of *H. atra* were used to test proximate composition. The moisture, ash,  
20 protein and crude fiber content were determined according to the Pearson's composition and analysis  
21 of foods [8].

##### 22 **2.2.1.1 Moisture content:**

23 Accurately, 1 g of samples were incubated in pre-heated oven at  $105^{\circ}\text{C}$ . the weight was measured  
24 after 3 hour of drying and the weight difference was calculated to obtain the moisture content.

##### 25 **2.2.1.2 Ash content:**

26 Accurately, 1 g of sample was incinerated in a muffle furnace at  $550-600^{\circ}\text{C}$  for 24 hours. The resulted  
27 ash was weighed and the percentage was calculated.

### 1 2.2.1.3 Total protein content

2 The Kjeldahl method was performed according to method 981.10 of the AOAC [9]. Approximately  
 3 50 mg of raw material was hydrolysed with 15 mL concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) containing two  
 4 copper catalyst tablets in a heat block (UDK 149 Automatic Kjeldahl Nitrogen Protein Analyzer) at 420  
 5 °C for 2 h. After cooling, distilled water was added to the hydrolysates before neutralization and  
 6 titration. The amount of total nitrogen in the raw materials were multiplied with the traditional  
 7 conversion factor of 6.25.

$$8 \quad \text{Nitrogen (\%)} = \frac{(\text{standard acid volume} - \text{ml blank}) \times \text{Nofacid} \times 1.4007}{\text{weight of sample (g)}}$$

$$9 \quad \text{Crude Protein (\%)} = \text{Total N (\%)} \times \text{Conversion factor (6.25)}$$

### 10 2.2.1.4 Total fat content:

11 Total fat content was determined using the Werner-Schmid method by Hill [10]. Briefly, 10 g portion of  
 12 the sample underwent digestion with HCl to facilitate the release of fat. The fat was subsequently  
 13 extracted using petroleum ether. Following the evaporation of the ether, the remaining residue was  
 14 weighed and calculated the fat content by using following equation.

$$1 \quad \% \text{ Fat in sample} = \frac{\text{Weight of fat in sample}}{\text{Weight of sample taken (g)}} \times 100$$

### 16 2.2.1.5 Carbohydrate content

17 Carbohydrate content was calculated by the difference of all other components measured [8].

$$18 \quad \% \text{ Total Carbohydrate} = [100 - \% (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash} + \text{Fiber})]$$

### 19 2.2.2 Lipid composition analysis of *Holothuria atra*

20 Determination of saturated, monosaturated, and polyunsaturated fatty acids with Eicosapentaenoic  
 21 acid (EPA), and Docosahexaenoic acid (DHA), content of these acucumber flesh was analysed with a  
 22 Gas chromatograph [11] (GCMS-TQGCHeadspace/Autosampler Trace 1300 Thermoscientific,  
 23 Industrial technology institute, Sri Lanka). Cholesterol content was measured by following the method  
 24 of Association of Official Analytical Chemists (AOAC) 994.10 [12] with a Gas chromatograph (GCMS-  
 25 TQGCHeadspace/Autosampler Trace 1300 Thermoscientific, Industrial technology institute, Sri  
 26 Lanka).

### 1        2.2.3    Vitamins analysis of *Holothuria atra*

2        The concentration of vitamin A was determined using analytical high-performance liquid  
3        chromatography (HPLC) (Methanol:acetonitrile 50:50 phase) following AOAC 2001.13 [13] method  
4        and Vitamin C was detected following the method AOAC 2012.22 [14] at the Industrial technology  
5        Institute (ITI), Colombo, Sri Lanka.

### 6        2.2.4    Mineral Composition analysis of *Holothuria atra*

7        Minerals; calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), selenium (Se),  
8        copper (Cu), iron (Fe), and manganese (Mn) were analysed by Inductively Coupled Plasma Mass  
9        Spectroscopy (ICP-MS). Briefly, 0.5 g of samples were digested with conc. HNO<sub>3</sub> (anal grade) at 200  
10       °C for 20 minutes followed by filtering and detection by ICP-Mass Spectrometer (Agilent 7900 ICP-  
11       MS, Residual analysis laboratory, Industrial technology institute, Sri Lanka).

### 12       2.3 Extraction of collagen from *Holothuria atra* body wall

13       Collagen extraction was carried out according to the method described by Yuniati and Sulardiano [15]  
14       with slight modifications. Briefly, coarsely ground sea cucumber samples (Panasonic AC300, Japan)  
15       incubated in distilled water (1:10 w/v) for 30 minutes with stirring. In the pre-treatment process,  
16       samples were mixed and stirred with 50% alcohol (1:2 w/v) for 30 minutes, washed with distilled  
17       water until the pH was neutral. Samples were immersed in HCl 0.1 M and 4 mM EDTA (1:10 w/v) for  
18       24 hours stirring continuously to maintain stable pH and reduce minerals. Followed by washing with  
19       distilled water, to eliminate non-collagenous proteins, samples were added to 0.1 M NaOH solution  
20       (1:10 w/v) and kept for 2 days, changing the NaOH solution in every 24 hours. Samples were  
21       thoroughly rinsed with cold distilled water until the rinsed water became neutral (pH 7.0). Remaining  
22       pellets were subjected to isolation of collagen by incubating in 0.5 M acetic acid (1:10 w/v) for 48  
23       hours with continuous stirring. Supernatant was separated by centrifugation (HERMLEZ 306) at 6000  
24       rpm for 20 minutes. The pellets were re-extracted with 0.5 M acetic acid for 24 hours, both  
25       supernatants were pooled, later NaCl was added to salt out collagen until the NaCl final concentration  
26       of the supernatant reaches 1 M. Precipitated collagen was separated by centrifugation at 2000 g for 15  
27       minutes and freeze-dried (ilShinBioBase FDS8512 freeze dryer).

### 1        2.3.1    **Calculation of yield of Acid Soluble Collagen (ASC)**

2        TheyieldofextractedASCwascalculatedbasedonthedryweightofstartingmaterialasper  
3        followingequation,

$$4 \qquad \text{Yield(\%)} = \frac{\text{Weight of lyophilized collagen (g)}}{\text{initial dry sample (g)}} \times 100$$

## 5        **2.4 Physico-chemical characterization of collagen**

### 6        2.4.1    **Determination of moisture content**

7        ThemoisturecontentwasdeterminedasperthethodologybytheAssociationofOfficialAnalytical  
8        Chemists[16]bydrying1gofASCat105°Cuntilaconstantweightwasobtained.The moisture  
9        contentwascalculatedbasedontheweightdifference.

### 10       2.4.2    **Determination of pH**

11       Approximately1gofcollagenwasdissolvedin70mlofdoubledistilledwaterandthepHwas  
12       measuredusingapHmeter(ConsortC6010Multi-parameteranalyser)[15].

### 13       2.4.3    **Attenuate Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)**

14       ASCsamplesweresubjectedtoATR-FTIRspectroscopyusingFTIRspectrometer(BrukerAlphaFT-  
15       IRspectrometer,DepartmentofMaterialScience,UniversityofMoratuwa).Thespectraintherange  
16       of600-4000cm<sup>-1</sup>withautomaticsignalgainwerecollectedin16scansataresolutionof4cm<sup>-1</sup>and  
17       wereratedagainstabackgroundspectrumrecordedfromthecleanemptycellat25°C.Analysis  
18       ofspectraldatawascarriedoutusingOMNICSpecta™datacollectionsoftwareprogram.

### 19       2.4.4    **UltraViolet (UV)-Vis absorptionspectra analysis**

20       Collagensolution(5mg/mL)waspreparedbydissolvingcollagenin0.5Maceticacid.Baselinewas  
21       setwith0.5Maceticacid.UV-VisabsorptionspectraofASCsampleswererecordedusingUV-Vis  
22       spectrophotometer(ModelThermoScientificGENESYS10SSeriesUV-Visspectrophotometer,  
23       InstrumentCentre,USJ)intherangeof190-600nm.

### 24       2.4.5    **Visualization of collagen under Scanning Electron Microscopy (SEM)**

25       TheinternalfibearchitectureofextractedcollagenwasobservedunderSEM(CARLZEISSEVO18  
26       ScanningElectronMicroscope,DepartmentofMaterialSciences,UniversityofMoratuwa).

### 1 3.RESULTSANDDISCUSSION

#### 2 3.2Nutritionalcompositionof *Holothuriaatra*

##### 3 3.2.1 Proximateanalysis

4 Accordingtothisstudy,proximateanalysiswascarriedoutforfreshseacucumbersamplescollected  
 5 fromMannar,SriLankawhichrecordedfortheirsttime.Themoistureamountoffoodcomponent  
 6 whichisaknownparameterisanindexofitswateractivity[17].Fresh bodywalls ofsea cucumber *H.*  
 7 *atra*resultedhigher moistureas83.2%andfoundtobeinaccordancewiththepreviousworkwith  
 8 severalseacucumberspecies,suchas *Holothuriascabra*(81.66%),*Holothuriaspinifera*(80.48%)  
 9 *Bohadschiasp.*(86.48%),*Bohadschiamarmorata*(84.65%)(Nishanthanetal.,2018)and *Holothuria*  
 10 *leucospilota*(84.52%)[18].Themoisturecontentcanbeinfluencedwithenvironmental,geographical  
 11 variations,behaviour,feedingandthecollectiontimeoftheyear[3].However,relativelylargeamount  
 12 ofmoistureindicatesthepotentialityofconcentratingthenutrientsthroughlossofwateror  
 13 dehydrationwhichis importanttoincrease theshelf-life whencommercializing[19] .

14 Ashcontentmaydependonthemineraldepositandotherorganicmatterinfleshwhichget  
 15 influencedbytheenvironmentandspecies[20].The ashcontentof *H.atra*flesh wasmeasured as  
 16 2.5%indicatingrelativelylowashcontentthanotherseacucumberspeciesreportedssuchas,  
 17 *Holothuriatubulosa*(5.13%),*Holothuriapolii*(7.85%)and *Holothuriamammata*(5.13%)byAydinetal  
 18 [21].

19 Theproteincontentisimportantforqualityandtextureofthemuscleofaquaticanimals.Theprotein  
 20 contentofthe *H.atra*fleshwasrecordedas10.2%basedontheweightindicatingahighvalue  
 21 comparedtootherspeciesas *H.polii*(8.66±1.2%),*H.tubulosa*(8.82±0.30%)and *H.mammata*  
 22 (7.88±0.3%) byAydinetal.[21].

23 Itwasobservedthatbodywallof *H.atra*has0.2%crudefibread2.0%fatcontentshowsthatsea  
 24 cucumberstendtohavenlowcontentofflipid.Thefatcontentofseacucumbersmightbeinfluenced  
 25 with several factors such as species, reproductivity, feed availability, feeding pattern and  
 26 environmentalconditionswhileittendstobehigherinconstanttemperaturesthanfluctuating  
 27 temperatures.However,fatisanimportantfactortobeconsiderednutritionallyduetoitsimportance  
 28 as anenergy sourceandrelevance formany otherimportant functionsin thehuman body[22].

1 Reported Carbohydrate content (2.1%) of the study was higher than several previous studies (0.86%  
2 in *Paracaudina australis*) [23], while the reported energy value of *H. atra* was 67 kcal/100g.

3

4

5

Table 1: Proximate analysis (% wet weight basis) of flesh of *H. atra*

Parameter	Weight (%)
Moisture Content	83.2
Ash content	2.5
Protein Content	10.2
Crude Fibre Content	0.2
Fat Content	2.0
Carbohydrate Content	2.1

6

7

### 8 3.2.2 Lipid profile analysis of *Holothuria atra*

9 Seacucumbers are believed to contain bioactive substances that assist in many physiological  
10 processes such as wound healing as they feed on bottom sediments enriched with branched chain  
11 fatty acids [24]. Therefore, the fatty acid composition of seacucumbers, especially PUFA will be of  
12 huge concern. Based on the research, total of saturated fatty acid (SFA) is more or less similar  
13 range with the total of monosaturated fatty acid (MUFA) and total of polyunsaturated fatty acid (PUFA).  
14 As per the previous studies, almost of seacucumber have shown higher amount of saturated fatty  
15 acid and Monosaturated fatty acid and lower content of Polyunsaturated fatty acid [25]. This result fits  
16 with Ridzwan et al. [25] which showed that saturated fatty acids were found dominated in *H. scabra*, *H.*  
17 *leucospilota* and *H. atra* as well. Further, there recorded low EPA value (0.05 g/100g) and absence of  
18 DHA as similarly recorded in the previous study of 4 seacucumber species; *S. horrens*, *H.*  
19 *leucospilota*, *H. atra* and *H. scabra* [25].

Table 2: Fatty acid profile of flesh of *H. atra*

Parameter	Amount
Saturatedfattyacid(g/100g)	0.4
Monosaturatedfattyacid(g/100g)	0.3
Polyunsaturatedfattyacid(g/100g)	0.1
Eicosapentaenoicacid(EPA)(g/100g)	0.05
Docosahexaenoicacid(DHA)(g/100g)	Not
detectedCholesterolcontent(mg/100g)	1.1

1

### 2 3.2.3 Vitamin(VitaminAandC)analysisofSeacucumberflesh

3 VitaminAandCwerenotdetectedin*H. atra*. Nevertheless, VitC(3.19mg/100g)hasbeendetected  
 4 in*H. scabra*whichbelongtothesamegenus[26]. Nutrient levelsolely dependson environmental  
 5 factors, season, location,type and sizeof the seacucumber species.

### 6 3.3Mineralanalysisof*Holothuriaatra*

7 Evaluationofmineralcompositionof*H. atra*ispresentedintable3. It hasbeenreportedinvarious  
 8 studiethatseacucumbersarealsoasourceofminerals, ingeneral, compositionisinfluencedbya  
 9 numberoffactorssuchasphysiologicalfactors, environmentalconditions, habitatandlifecycle[27].  
 10 Sodium(Na)wasthelargestinthecompositionfollowedbycalcium(Ca), magnesium(Mg),  
 11 potassium(K), zinc (Zn), iron (Fe), copper (Cu), selenium (Se) and manganese (Mn).

12 Table3:Mineralcompositionof*H. atra*flesh

Mineral	Amount(mg/kg)
Calcium(Ca)	1200
Magnesium(Mg)	1100
Sodium(Na)	4200
Potassium(K)	629
Zinc (Zn)	5.9
Selenium(Se)	2.1
Copper(Cu)	4.0
Iron(Fe)	5.3

Manganese(Mn)

1.7

### 1      **3.4 Calculation of yield of ASC**

2      Acid-Solubilized Collagen (ASC) extraction for the body wall of sea cucumbers (*Holothuria atra*)  
 3      resulted a white colour cotton wool like solid with a yield of 0.95% based on the dry weight basis. This  
 4      is higher than the yield recorded (0.88%) in the previous study conducted on the same species by  
 5      Yuniati and Sulardiono [15]. A low yield can be resulted due to covalent crosslinking in the peptide  
 6      region, thus reducing the collagen solubility, while the level of collagen yield can be increased after  
 7      peptide digestion using pepsin enzyme while improving the collagen extraction ability [28].

### 8      **3.5 Physico-chemical characterization of collagen**

#### 9      **3.5.1      Determination of moisture content and pH**

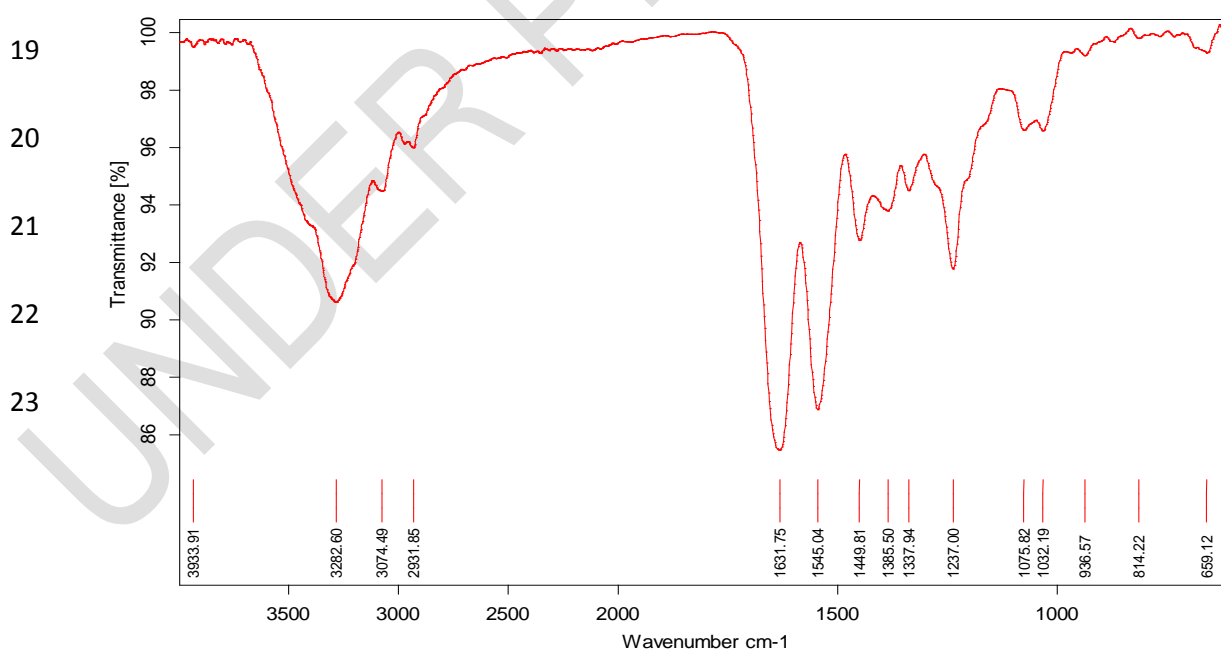
10      The moisture content of the collagen from *H. atra* was measured as 7.246%. The affinity of collagen  
 11      and water is highly affected by collagen organization [29]. Further, the pH value plays a crucial role in  
 12      formulation of collagen, especially for commercialization purposes [30]. The pH of the *H. atra* in the  
 13      current study was measured as 3.95 and this might be influenced by the acid concentration used in  
 14      the extraction. A similar result has been reported in a previous study by [31], which used PSC (Pepsin  
 15      Soluble Collagen) extraction method. As per previous studies, the best pH range of commercial  
 16      collagen for cosmetics was reported between 3.8 to 4.7 [30].

#### 17      **3.5.2      Attenuate Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)**

18      The FTIR spectra of extracted collagen sample from *Holothuria atra* is illustrated in the figure 1.  
 19      Major functional groups of collagen were identified in the extracted *H. atra* collagen sample and  
 20      confirmed by the presence of characteristic bands, amide A ( $3282.60\text{cm}^{-1}$ ), amide B ( $2900-3080\text{cm}^{-1}$ ),  
 21      amide I ( $1631.75\text{cm}^{-1}$ ), amide II ( $1330-1545\text{cm}^{-1}$ ), amide III ( $1075-1240\text{cm}^{-1}$ ). Amide A band is resulted  
 22      from stretching vibrations of N-H group. The range  $3400$  to  $3440\text{cm}^{-1}$  is commonly attributed to the  
 23      N-H stretching vibration and when the N-H stretching is involved with hydrogen bonds, the amide A  
 24      peak is shifted to lower wavenumber [32]. Therefore, the resulted peak of amide A close to  $3300\text{cm}^{-1}$   
 25      in this study indicates presence of H-bonds in the N-H stretching of the extracted collagen sample.  
 26      The asymmetrical shape of the amide A peak observed further reveals presence of some amount of  
 27      water in the collagen sample. As per Susi and Ard [33], absorption band for bound water should be  
 28      overlapped with amide A illustrating a low-frequency shift of  $\sim 100\text{cm}^{-1}$  for the  $\text{H}_2\text{O}$  absorption band.

1 The observed peaks of Amide B were identified and attributed at  $3070.49\text{ cm}^{-1}$  for  $\text{CH}_2$  asymmetric  
 2 stretching and at  $2931.85\text{ cm}^{-1}$  for  $\text{CH}_2$  symmetric stretching which were reported by Abe and Krimm  
 3 [34] in the similar range. The peak appearing at  $1631.75\text{ cm}^{-1}$  was assigned to an amide I band which is  
 4 associated with stretching vibrations of the carbonyl group ( $\text{C}=\text{O}$  bond) along the backbone of the  
 5 polypeptide chain. The amide I peak is usually found in the range from  $1600$  to  $1700\text{ cm}^{-1}$  and the  
 6 lower side shifted frequency observed was due to the high number of H-bonds of the carbonyl of an  
 7 amide group [32].

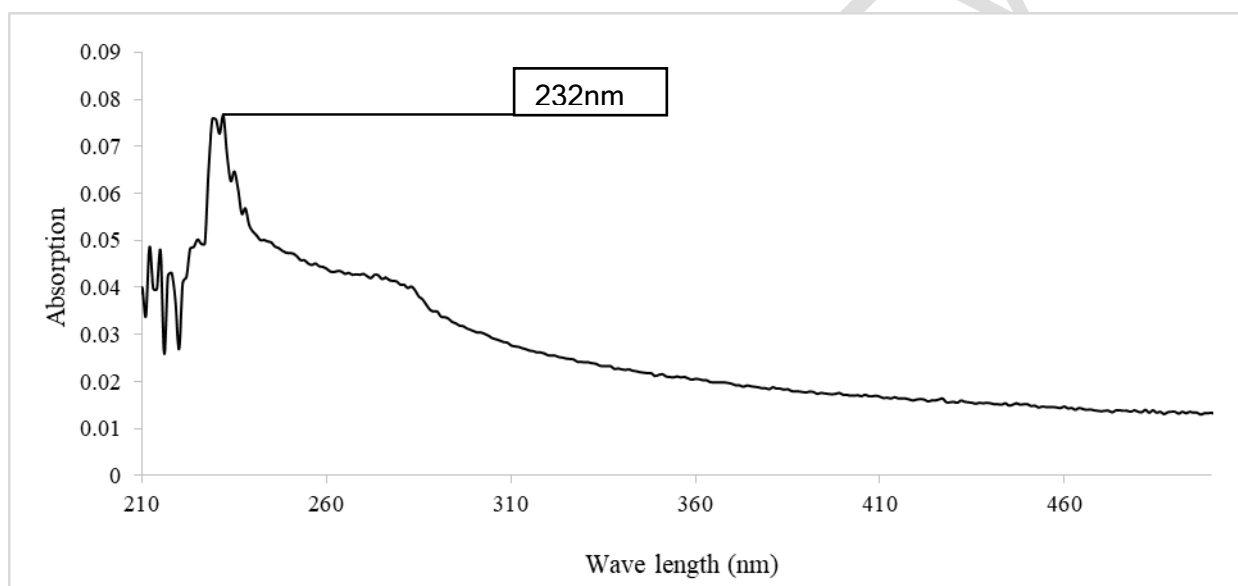
8 Amide II peak was observed at  $1545.45\text{ cm}^{-1}$  was attributed to the N-H bending vibration strongly  
 9 coupled to the C-N stretching vibration of protein amide groups which are usually observed in the  
 10 range  $1530$ - $1550\text{ cm}^{-1}$  [35]. Amide II minor bands at lower frequencies were also resulted in the study.  
 11 The peak at  $1449.81\text{ cm}^{-1}$  was attributed to  $\text{CH}_2$  bending and the peak at  $1385.50\text{ cm}^{-1}$  was derived  
 12 from  $\text{COO}^-$  symmetrical stretching. The peak at  $1337.94\text{ cm}^{-1}$  was observed and attributed to the  
 13 wagging vibration of the proline side chains found in the type I collagen of body tissues [35]. Amide III  
 14 peaks attributed to the N-H bending coupled with C-N stretching and C-O stretching were detected at  
 15  $1237.00\text{ cm}^{-1}$  and  $1075.82\text{ cm}^{-1}$  respectively. Moreover, functional group analysis with FTIR confirm  
 16 the preserved triple helix structure in the extracted collagen sample from *Holothuria atra* with amide  
 17 III/II ratio of  $0.85$  which is approximately equal to  $1.0$ . These similar results were reported previously  
 18 for *Stichopus japonicus* and *Holothuria parva* [28].



24 **Fig1.** The FTIR spectrum of acid-soluble collagen from *Holothuria atra* (Model Thermo Scientific Nicolet S10 FT-IR spectrometer, Instrument Centre, USJ)

### 1        3.5.3    UV–Vis absorptionspectra analysis

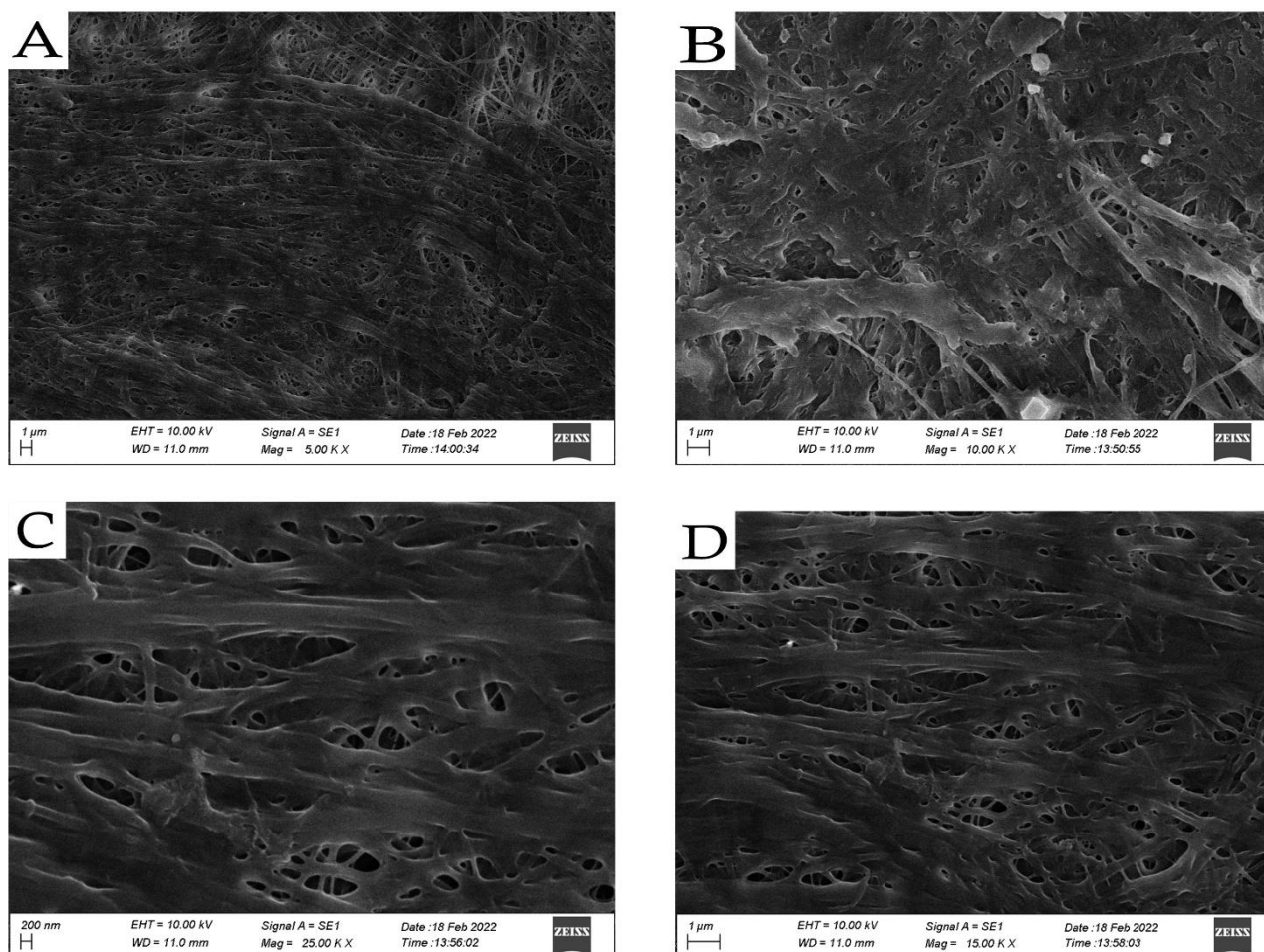
2        The UV-Vis spectrophotometric analysis was conducted to determine collagen's type and purity based  
 3        on the absorption at a pre-identified wavelength. The UV-Vis spectra of extracted collagen from  
 4        *Holothuria atra* (Figure 2) exhibited a maximum absorbance at 232 nm. As per the observed  
 5        maximum absorbance at 232 nm, it identified the presence of type 1 collagen in the tested sample.  
 6        Further, the absorbance peaks observed between 200–220 nm were attributed to the collagen peptide  
 7        bonds. The aromatic side chains contained in collagen absorb light in the 240–300 nm region resulting  
 8        several peaks at 280 nm (tryptophan), 275 nm (tyrosine), 258 nm (histidine, and phenylalanine) [31].  
 9        However, the no obvious absorption observed at around 250–280 nm indicating the fact that the  
 10        extracted collagen is in high purity.



18        **Fig2.** The ultraviolet absorption spectrum of ACS (Model Thermo Scientific GENESYS 10S Series UV-  
 19        Vis spectrophotometer, Instrumental Centre, USJ)

### 20        3.5.4    Scanning Electron Microscopy (SEM) analysis

21        Figure 3 shows the scanning electron microscope (SEM) images of collagen from *Holothuria atra*. It  
 22        shows bundles of circumference fibrils networks, having irregular and dense pleated appearance with  
 23        a loose and porous structure which described by Saallah et al. [31].



**Fig3.** SEM Images of ASC from *Holothuria atra*. (A) 5kx; (B) 10kx; (C) 25kx (D) 15kx (CARLZEISSEVO18 Scanning Electron Microscope, Department of materials sciences, University of Moratuwa)

#### 4. CONCLUSION

This study presents nutrition profile and collagen characterization of *Holothuria atra* body wall collected from North-west coast, Sri Lanka for the first time. It is rich with various nutrients. Further, Collagen was extracted from *Holothuria atra* body wall using the acid base extraction method which can be considered as a low yield. However, the collagen was characterized as type I collagen with a unique structure. Application of collagen from *Holothuria atra* in industry is questionable due to the low yield thus an alternative extraction method or development of a product with low yield is required in the future directions.

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