Application of Near-Infrared spectroscopy and chemometric (PCA) in variety holothuria atra and holothuria scabra in Simeuleu, Aceh province

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Abstract. As the largest maritime country globally, Indonesia has a variety of potential marine biota that can be explored as a source of nutrition and raw materials for medicine. As one of the marine biota, Sea cucumbers have been widely recognized by the public as a medicine for treatment from generation to generation. With the breadth and variation of Indonesian waters' characteristics, it is possible to have differences in the nutritional and bioactive content of various types and sources of sea cucumber. This study analyzes the compounds in Holothuria atra, and Holothuria scabra dried sea cucumbers using Near-Infrared Spectroscopy (NIRS). Spectrum data of dried sea cucumber samples were obtained from the absorbance spectrum in the wavelength range of 780 nm - 2500 nm with a resolution of 0.02 nm and 32 scans. The spectrum data is then projected onto principal component analysis (PCA) to extract data and observe similarities between the two samples. The results showed that there was no difference between the Holothuria atra and Holothuria scabra dry samples in terms of the spectral data showing their molecular bonds. However, the essential chemical structure related to the content of sea cucumbers is vibrating in the Near-infrared region, especially in the waves 1147, 1310, 1452, 1667, 1819, 1920, and 2235 nm, indicates the presence of molecular bonds C-H, O-H, C-O, C-H-O, N-H, and S-H. Based on the results obtained, it can be concluded that NIRS has the potential to analyze and determine several quality attributes of sea cucumber samples with further calibration modeling.

Introduction

Sea cucumber has a high nutritional content and pharmacological effects, making it a potential food and medicinal source [1]. As a food source, sea cucumber has been shown to contain high protein, low fat, and high levels of essential minerals [2]. Various active compounds in this species are thought to be capable of being a therapeutic breakthrough in various diseases [3]. Also, sea cucumbers have high economic value on the world market and are a valuable export commodity. Therefore, as the largest
archipelagic country, Indonesia has the potential to develop sea cucumber processing as food, medicinal ingredients, and export commodities.

Sea cucumbers have been known for a long time to treat hypertension, asthma, rheumatism, impotence, and constipation by the Chinese and other Asian nations [4]. Various chemical constituents have been extracted from these marine biota ranging from simple ones, such as polypeptides, to complex ones such as polyether [5]. Various phenolic compounds found in sea cucumbers include chlorogenic acid, pyrogallol, coumaric acid, and catechins. Experts have noted that there are 1,400 species of sea cucumber scattered throughout the world, and 55 of them are found in Indonesian waters, so there may be differences in the content of nutrients and other bioactive compounds [6]. Research conducted in the Aceh Besar area and Simeulue shows various types of sea cucumbers [7].

Sea cucumbers are usually processed into a dry product known as beche-de-mer. Beche-de-mer qualities are classified as high, medium, or low based on species, shape, odor, color, and body wall thickness. Classification based on physical property has a weakness because it depends on panelist abilities that are possibly different from one to others. Instrumental methods can be more accurate by providing more detailed information such as chemical compounds, property value, and even quantitative data.

In the application of organic material, the use of a spectroscopy instrument is a well choice. It has been widely and accurately applied on the identification of organic samples, such as identification of protein, lipid and fish meat with Uv-Vis [8–10], food and coal by laser [11,12], leaf tissue with image processing [13], bone and polysaccharide with FTIR[14,15], secondary metabolites and fatty acid with mass spectrometry [16–19], etc.

In the purpose of the cucumbers sample, fatty acid profile are identified as a basis in its quality determination. The use of FTIR and polymerase chain reaction (PCR) have shown a precise and reliable result. However, both techniques are complicated in the case of time-consuming and sample pre-treatment process. NIRS is known to work based on the theory of electromagnetic radiation interaction from the biological samples used. Many studies have used NIRS because of its ease in analyzing chemical properties or quality without special sample preparation. It is environmentally friendly because there is no need to use chemicals; the process is fast and does not damage the sample. Besides, NIRS also can predict and determine the desired quality or nutrient parameters by using spectral data simultaneously[20,21].

Near-infrared spectroscopy is an analytical technique that utilizes infrared light rays, which have a wavelength range of 780-2500 nm. In this area, vibrations of the -CH, -OH, -SH, and -NH bonds generally occur. Thus, near-infrared can be used to analyze the chemical content of the organic matter that has these bonds. NIR spectroscopy can guarantee the quality of both raw materials and finished products with easy sample preparation. Analysis using near-infrared also has several other advantages, including non-destructive, accurate, fast analysis time, and does not require chemicals[22]. So the use of near-infrared spectroscopy (NIRS) can be a good option to simplify the time and eliminate the treatment.

Regardless of a convenient analysis offered by this spectroscopy method, a spectra interpretation difficulty is still found. So, a statistical application, chemometric, is required to solve this problem. The combination of instrumental and chemometric measurements is a perfect choice for reliable and straightforward procedures [23–25]. One of the chemometric methods that have recently applied in several analytical works is PCA. This method can classify complex data based on the similarity or difference pattern one data to others[26–28]. This benefit is useful in the purpose to determine the cucumber samples into certain grade quality. Therefore, the use of NIRS combined with PCA in this research can potentially be methods of checking the contents and classification of various sea cucumbers quickly and easily. Based on the above reasons, the authors intend to assess whether NIRS can be used to distinguish the chemical content between Holothuria atra and Holothuriascabra.
Materials and methods

1.1. Sample source
The sea cucumbers used in this study were dried types of Holothuria atra and Holothuria scabra obtained in Simeulue waters, Aceh, Indonesia. Holothuria atra and Holothuria scabra were identified based on morphology to determine the type of species in the Marine Biology Laboratory, Faculty of Marine and Fisheries. On the other hand, near-infrared spectra data of studied samples were acquired and measured at the Laboratory of Instrumentation, Dept. of Agricultural Engineering, Universitas Syiah Kuala.

1.2. Infrared spectrum data
Equipment used: Portable sensing device Near-infrared spectroscopy (PSD-NIRS), i16 coupled with photodiode array sensors and programmable logic controller. Moreover, Spectra data were analyzed using principal component analysis using the CAMO Unscrambler X 10.3 (NC license, Olso Norway).

Spectrum data of dried sea cucumber samples were obtained from the absorbance spectrum in the wavelength range of 780 nm - 2500 nm with a resolution of 0.02 nm and 32 scans. Optical amplification and correction are also used to ensure smooth acquisition and eliminate spectrum interference during acquisition. The absorbance spectrum data were then projected onto principal component analysis (PCA) as a chemometric method for qualitative analysis to extract data and observe the similarities between the two types of samples. PCA classification method works by extracting spectrum data by entering the absorption data in a new projection matrix called main components (PCs). To get a strong classification, cross-validation is applied by using the non-iterative partial least square.

Result and discussion
The NIR spectra of Holothuria atra and Holothuria scabra are shown in Figure 1. From the results of the NIRS absorbance examination in the two species, visualization of the NIR spectrum did not show any differences between Holothuria atra and Holothuria scabra, where the two spectra appeared to coincide (not separate). This is in line with some of the previous studies which state that sea cucumbers have a lot of nutritional content such as various types of vitamins, high in minerals, especially calcium. Many research has proven that the main components of various sea cucumbers are collagen and polysaccharides, which have been studied to have various biological activities such as anti-inflammatory, anti-thrombotic, anti-tumor, and wound healing properties for various types of sea cucumbers. The secondary metabolites of sea cucumbers are associated with the presence of saponins, chondroitin sulfates, glycosaminoglycan polysaccharides, and essential fatty acids [29,30].

![Figure 1. The NIR spectra of holothuria atra and holothuria scabra.](image)

The two species optimum wavelengths in Figure 2 show the presence of absorption and strain over several wave ranges. The NIR spectrum in the 780 nm - 2500 nm wave range shows vibrations at 1147nm, 1310 nm, 1452 nm, 1667 nm, 1819 nm, 1920 nm, and 2235 nm wavelengths. This vibration indicates that NIRS noted the presence of an electromagnetic spectrum as a reaction to the presence of
O-H, C-H, C-O, N-H, C-H-O, and C-H-O-S groups and correlates with the specific chemical compound in sea cucumbers such as protein, fatty acids, and sulfates. This indicates the possible amino acid content, fatty acids, and aromatic compounds at overtones 1 and 2. Vibration in the 1819 nm region indicates sulfate groups' presence, while carboxylate groups are seen at 2235 nm [31].

![Loading plot derived from PCA analysis to determine optimum wavelength vibration of the group compound in holothuria atra and holothuria scabra.](image)

The molecular bonds found in Holothuria atra and Holothuria scabra and adjusted for the distribution of organic bonds in NIRS electromagnetic waves can be seen in Table 1.

| Wavelength (nm) | Chemical Bonds               |
|-----------------|------------------------------|
| 1147            | CH, CH₃CH₃                   |
| 1310            | CH, CH₃CH₃                   |
| 1452            | H₂O, ROH                     |
| 1667            | ArCH, CH, CH₂CH₃             |
| 1819            | SH                           |
| 1920            | H₂O                          |
| 2245            | NH₂, CHO, CH, CH₂, CH₃       |

These results align with other studies that stated that sea cucumbers contain lots of fatty acids, collagen-rich protein, and amino acids [32]. Glycosaminoglycan molecules such as chondroitin sulfate are allegedly present in sea cucumbers as components of the extracellular matrix found in various connective tissues such as cartilage, bone, skin, ligaments, and tendons. Giving chondroitin sulfate to patients with osteoarthritis can reduce pain and also improve joint function [33,34]. Meanwhile, the presence of CH, CH₂, and CH₃ groups indicates the possibility that triperpenoid compounds such as sequelants have been extensively studied as anticancer agents because they exhibit cytotoxic activity in certain types of cancer cells [35].
Figure 3. PCA analysis of holothuria atra and holothuria scabra.

From Figure 3, the main information derived from the PCA analysis results, there are no differences between Holothuria atra and Holothuria scabra. This means each sample can be replaced for their advantage purposes. Moreover, It also showed that chemometrics, utilizing the PCA method, can distinguish samples based on their origins. Loading plot, the PCA also mentioned that relevant and essential wavelengths are closely associated with several respective chemical constituents of sea cucumber samples.

Furthermore, to find out whether the content of chemical compounds on all sides of each sample's body is the same or different, the authors tried to illuminate 4 different sides of the sample, from the upper and lower ends and two points from the midsection. The results obtained were analyzed using principal component analysis (PCA), which shows the euclidian distance away from each radiation position. This indicates the possibility of certain types of compound content in the upper, lower, and middle ends of the sea cucumber's body, but the amount cannot be determined.

However, as a whole, based on the PCA results in Figure 3, it can be seen that there are similarities in the content of compounds between Holothuria atra and Holothuria scabra both in the upper, middle, and lower parts of the body of the two species. Thus, the main information obtained from the PCA analysis results, there is no difference between Holothuria atra and Holothuria scabra. PCA can also be employed to discover leverage and outlier data of spectra before spectra enhancement and correction. Consequently, it can provide more robust classification overview. This will be performed later on a further step in combination with discriminant analysis to classify samples.

Conclusions
Based on principal component analysis (PCA) results, there are relatively no differences in chemical compounds between the two samples of sea cucumbers. The PCA method attempted to extract and classify spectral data based on their similarities and characteristics of sea cucumber samples. However, the important chemical structures associated with the molecular bonds of C-H, O-H, C-O, C-H-O, N-H, and S-H produce vibrations at 1147, 1310, 1452, 1667, 1819, 1920, and 2235 nm. The obtained results indicate that NIRS, combined with the PCA data analyses, were able to extract buried information inside spectra data. The respective information is that there are no differences between Holothuria atra and Holothuria scabra. This means each sample can be replaced for their advantage purposes. Moreover, It also showed that chemometrics, utilizing the PCA method, can distinguish samples based on their origins. Loading plot, the PCA also mentioned that relevant and important wavelengths are closely associated with several respective chemical constituents of sea cucumber samples.
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