Effects of Sun and Oven Drying on The Physicochemical Composition of Indonesian Sandfish (*Holothuria scabra*)

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Abstract

Sandfish (*Holothuria scabra*) is a type of sea cucumber with high economic value. Sandfish are potentially used as food, pharmaceutical, and cosmetic ingredients. This research aimed to determine the effects of sun and oven drying on the physicochemical composition of sandfish collected from Pesawaran Waters, Lampung, Indonesia. The physicochemical composition includes the proximate composition, saponin content, collagen content, and metabolite profiles. The sea cucumbers were divided into two groups; the first group was boiled for 30 minutes, whereas the second group was unboiled. The sea cucumbers were oven-dried (temperature 60 °C for 24 h) and sun-dried (3 days from 08:00 a.m. until 5:00 p.m.). Fresh sandfish (without drying) were used as control samples. The dried and fresh sandfish were analyzed for their proximate composition (moisture, ash, fat, protein, and carbohydrates) and collagen content. The dried and fresh sandfish were extracted by maceration using ethanol. The extract was analyzed for its saponin content and metabolite profiles. The results showed no significant differences (p>0.05) between oven-dried and sun-dried sandfish regarding their proximate composition and collagen content. However, the saponin content in boiled sandfish extract was significantly different (p<0.05) from the saponin content in sandfish extract that was unboiled. The highest saponin content of all treatments was in the boiling and oven-drying treatment (N1P2), i.e., 7.418 mg quillaja/10mg extract. The Fourier Transform Infrared (FTIR) profile indicated that the functional groups in the sandfish extract did not change during the drying process. The findings indicate that oven and sun drying do not negatively affect the physicochemical composition of the sandfish, especially its bioactive compounds, i.e., saponin and collagen.

Keywords: collagen, drying, *Holothuria scabra*, metabolite, profile saponin

Introduction

Sea cucumbers, classified as invertebrates within the echinoderm phylum under the Holothuroidae class, have a longstanding history of utilization in Asian and Middle Eastern cuisines and traditional medicinal practices. These marine organisms boast a rich nutritional profile, featuring high-quality protein, amino acids, vitamins, minerals, and fatty acids (Bordbar et al., 2011). Moreover, sea cucumbers are abundant in various bioactive components, including peptides, polyunsaturated fatty acids, saponins, chondroitin sulfate, glycosaminoglycans, and sulfated polysaccharides (Sroyraya et al., 2017; Ramalho et al., 2020). The reported benefits of sea cucumbers encompass a wide range of health-promoting properties such as anticancer, anti-inflammatory, antioxidant, immunomodulatory, anticoagulant, wound healing, hypoglycemic, and hypolipidemic activities (Khotimchenko, 2018; Wen et al., 2016;). Given their status as a source of both bioactive compounds and high-quality nutrients, sea cucumbers exhibit significant potential for development as ingredients in food, cosmetics, and pharmaceuticals.

The sandfish (*Holothuria scabra*), a variety of sea cucumbers commonly found in Indonesian waters, holds considerable economic significance (Yusron, 2004). Notably, successful cultivation of sand sea cucumbers has been achieved, presenting a promising avenue for their sustainable utilization as raw materials (Widianingsih, 2019). Numerous studies have investigated the bioactivity of *H. scabra*. Kim et al.

(2016) and Nobsathian et al. (2017) demonstrated its antioxidant properties and anti-wrinkle and melanogenesis inhibitory activities. Additionally, Wong et al. (2023) and Han et al. (2012) highlighted *H. scabra*'s potential as an antioxidant, antiglycation agent, and anticancer substance.

The primary constituent among secondary metabolite compounds in sea cucumbers is saponin, a crucial group of natural substances initially observed in higher plants. Triterpene glycosides, also referred to as holothurian or saponins, constitute secondary metabolites synthesized by sea cucumbers belonging to the class Holothuroidea (Caulier et al., 2011). Approximately 135 saponins from the Holothuria genus have been characterized (Puspitasari et al., 2022). Sea cucumber saponins exhibit noteworthy properties as immunostimulants, antifungals, antioxidants, and anticancer agents (Aminin et al., 2006; Li et al., 2013). In addition to possessing elevated saponin levels, the dermal structure of sea cucumbers is characterized by substantial quantities of collagen (Ghufran et al., 2010). Collagen serves numerous roles in restoring compromised tissues (Park et al., 2012), making it extensively employed in the cosmetics industry. Collagen fibres are used in cosmetic products due to their antioxidant properties (Xu et al., 2010).

One of the challenges associated with developing bioactive compounds from sea cucumbers pertains to the processing of raw materials, specifically the drying process, which influences the stability of bioactive compounds and other valuable constituents in sea cucumbers (Ram et al., 2017). Sea cucumbers are commonly processed into a dried product known as *beche-de-mer*, with traditional methods involving salting, repeated cooking, and sun drying for 2-3 days (Duan et al., 2007). Various drying methods are employed, including sun drying or mechanical drying. Sun drying is a straightforward and cheap method, but it is time-consuming compared to oven drying, which efficiently reduces water content in substantial quantities over a short duration (Muller et al., 2006). Mechanical drying also accelerates the drying process compared to sun drying, which takes approximately 3-4 days. Drying aims to eliminate water content in sea cucumbers, enabling long-term storage (Adawiyah, 2007). Handling dried sea cucumbers in industrial contexts is more convenient and cheaper than dealing with fresh sea cucumbers. Purcell (2014) emphasized the importance of employing appropriate processing techniques to produce high-quality dried sea cucumbers. Proper processing necessitates consideration of the sea cucumber type, post-harvest handling, and the processing method. This research investigated the impact of sun- and oven-drying on the physicochemical composition of sandfish, which covers proximate composition, saponin and collagen content, and their metabolite profiles.

Material and Methods

Collection of *Holothuria scabra*

Fresh *Holothuria scabra* (sandfish with local name of teripang pasir) were obtained from Pesawaran Waters, Lampung, Indonesia, on October 15th – 20th, 2022. Their internal organs were removed prior to preservation. Cleaned sandfish were packed in plastic bags, iced, and transported to the Integrated Oceanographic Research Laboratory, National Research and Innovation Agency, Jakarta, Indonesia. Sandfish were then stored at -20 °C before further analysis.

Sample Preparation

The defrosted sandfish were weighed (200-300 g) and cut into small pieces (± 2-3 cm). Two treatments were given, i.e., boiling for 30 min (100 °C) in water (group 1) and without boiling (group 2). Group 1 sandfish were ready to be dried after boiling and draining. Group 2 sandfish were treated by lysis with osmotic shock, according to O'Connor et al. (2020). The defrosted sandfish (50 g) were cut into small pieces (± 2-3 cm) and stored in the freezer at -80 to -70 °C for 12 h. After thawing, they were soaked in deionized water and sonicated for one hour at 30-40 °C. The sandfish were then drained and ready for drying. Both methods, i.e., boiling and lysis with osmotic shock, aimed to remove residual salts/minerals and prevent odor and spoilage in further treatments.

Drying Process

The sandfish in groups 1 and 2 were exposed to the sun and oven-drying. They were sun-dried for three days (08:00 – 17:00) and oven-dried (Memmert GmbH, Germany) for 24 h at 60 °C (Nursid et al., 2022).

Proximate Analysis

Analysis of ash, protein, and lipid was carried out following the Association of Official Analytical Chemists (AOAC) method (AOAC, 2016). The ash content was determined after burning the sample in a furnace set at 550 °C. The Kjeldahl method determined the protein content using a nitrogen conversion factor of 6.25.

The fat content was determined using the Soxhlet extraction method, whereas the total carbohydrate was determined by a different method.

Extraction of Active Compounds

The active compounds of sandfish were extracted by the maceration method. The dried and fresh sandfish (control) were placed in ethanol solution (analytical grade, 99.8%) with a ratio of 1:1 (w/v) for 24 h at room temperature, and this process was repeated three times. The filtrate was filtered using Whatman filter paper and concentrated by a vacuum rotary evaporator (Buchi, Swiss). The concentrated extract was further dried in a vacuum concentrator until dried extract was obtained.

Analysis of Saponin Content

The saponin content was analyzed using the vanillin-sulfuric acid colorimeter method, adapted from the literature (Bondoc et al., 2013; Dewi et al., 2023). A total of 20 µL of sandfish extract (10 mg/mL) was added to a solution of 5% (w/v) vanillin in bi-distilled water, to which 500 µL of 72% sulfuric acid was added. The solution was incubated for 10 min at 60 °C and cooled before measuring its absorbance at 540 nm in a spectrophotometer (Thermo Scientific, USA). The concentration series of Quillaja bark saponin (Sigma-Aldrich, Germany) were prepared (0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/mL) and used to generate a standard curve.

Analysis of Collagen Content

Determination of collagen content was carried out using the method of Brown et al. (2001) and Dewi et al. (2023), with slight modifications. A total of 30 mg of dried sandfish was hydrolyzed using 1 mL of 50% HCL and incubated for four hours at 110 °C. The solution was then neutralized by adding 4 mL of 1 M NaOH. The particles formed were removed by centrifugation at 6000 rpm for 10 min. The hydrolyzed solution was diluted 20 times before analysis. A total of 50 µL of the diluted solution was mixed with 100 µL of chloramine T solution (300 mg in 500 µL oxidation buffer)) and 100 µL of Ehrlich's reagent (6 g p-DAB, 52 mL isopropanol and 16 mL 50% perchloric acid). The mixture was incubated at 60 °C for 45 min, and then the absorbance was measured with a spectrophotometer (Thermo Scientific, USA) at a ë of 570 nm. Collagen standard from bovine achilles tendon (Sigma-Aldrich, Germany) was used to build a standard curve (0 – 10 mg/mL). The stock solution of collagen standard was prepared by subjecting 10 mg of collagen standard to the same treatment series as the dried sandfish.

FT-IR Analysis

The infrared spectra of the sandfish samples were recorded from 4000 to 400 cm^{-1} by the Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) technique, using a Bruker TENSOR II FT-IR spectrometer coupled with a diamond ATR device. The sandfish extract was diluted, and 15 ìL of this sample was directly applied to the ATR surface and analyzed. FTIR spectra were interpreted based on Pretsch et al. (2000).

Data Analysis

The sandfish in this study were subjected to five treatments, namely boiled and sun-dried sandfish (N1P1), boiled and oven-dried sandfish (N1P2), unboiled and sun-dried sandfish (N2P1), unboiled and oven-dried sandfish (N2P2), and fresh (unboiled and without drying) sandfish as the control. All experiments for each treatment were carried out in two replicates, and the data obtained from two replications were analyzed using One-way analysis of variance (ANOVA) followed by the Tukey test. Statistical analysis was performed using SPSS (Version 26), and a significance level of 95% (p<0.05) was applied.

Results and Discussion

Yield

The yield of dried sandfish was between 9.96 and 10.20%, whereas the dried extract was 0.0188 – 0.0343%. The dried sandfish or extract yield was not significantly different (p>0.05) between drying techniques. However, it was significantly different (p<0.05) when it was compared with the yield of control (fresh sandfish) (Table 1). The extract from dried sandfish has a lower yield than the extract from fresh sandfish. The flesh of dried sandfish was harder and denser than fresh sandfish, making the extraction process of dried sandfish more complex than the fresh sandfish. This might hinder ethanol from diffusing into all parts of the dried sandfish. As a result, fewer bioactive compounds could be extracted by the ethanol from the dried sandfish than from the fresh sandfish. This study's results align with Sari et al. (2014), which showed a higher extract yield from fresh *H. edulis* than dried *H. edulis*. In addition, the selection of solvent

Table 1. Yield of sandfish (*H. scabra*)

Treatment	Dried sandfish (%)	Sandfish extract (%)
Fresh		1.0445± 0.936[a]
N1P1	10.20 ± 0.280 [a]	0.0299± 0.006 [b]
N1P2	10.08 ± 0.101 [a]	0.0343± 0.004 [b]
N2P1	10.11 ± 0.772 [a]	0.0188± 0.001 [b]
N2P2	9.96 ± 0.237 [a]	0.0236±0.005 [b]

Note: N1P1 (boiled, sun drying), N1P2 (boiled, oven drying), N2P1 (unboiled, sun drying), N2P2 (unboiled, oven drying). Treatments that do not share the same letters in the same column significantly differ by ANOVA (p<0.05).

Table 2. Proximate composition of fresh, sun-dried, and oven-dried sea cucumber *H. scabra* based on % dry weight (% DW).

Treatment	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Fresh	87.64 ± 1.22[a]	6.02 ± 1.93[a]	5.11 ± 0.23[a]	0.03 ± 0.0[a]	1.82 ± 0.74[a]
N1P1	8.59 ± 0.38[b]	38.19 ± 4.20[b]	38.75 ± 3.69[b]	1.15 ± 0.007[b]	13.30 ± 0.12[b]
N1P2	9.52± 0.49[b]	35.29 ± 2.05[b]	46.44 ± 4.20[b]	1.10 ± 0.39[b]	7.63 ± 5.38[b]
N2P1	8.41 ± 0.09[b]	33.65 ±0.54[b]	40.91 ± 3.70[b]	1.05 ± 0.14[b]	15.97 ± 5.11[b]
N2P2	9.69± 0.91[b]	38.69 ± 3.95[b]	40.45 ± 1.47[b]	0.62 ± 0.08[b]	10.53 ± 6.26[b]

Note: N1P1 (boiled, sun drying), N1P2 (boiled, oven drying), N2P1 (unboiled, sun drying), N2P2 (unboiled, oven drying). Treatments that do not share the same letters in the same column significantly differ by ANOVA (p<0.05).

for extraction seems to affect the yield. Karlina et al. (2011) reported a yield of 1.3% from fresh *H. scabra* when methanol was used as a solvent. Further study on the yield optimization with different solvents might be required.

Proximate Composition

Table 2 presents a proximate analysis of the sandfish. The proximate composition of sun-dried and oven-dried sandfish significantly differs from that of fresh sandfish. Still, there were no significant differences between the proximate composition of the sun-dried sandfish and the oven-dried sandfish.

The moisture content of dried *H. scabra* was between 8.41% and 9.69%, somewhat in line with the result of Martoyo et al. (2006). The study showed that the moisture content of sandfish dried using a mechanical dryer was 8.9%, whereas Ibrahim et al. (2015) reported that dried *H. scabra* originating from the Sudanese Strait was 6.20%. The ash content of dried *H. scabra* was in the range of 33.65–38.69%. Ibrahim et al. (2015) reported that the ash content of dried *H. scabra* originating from the Sudanese Strait was 31.67%. The ash content is mainly attributed to the minerals contained in a material. High ash content in a sample means the sample has high mineral content (Nielsen, 2010).

The protein content of dried *H. scabra* was 38.75 - 46.44%, whereas the control (fresh sandfish) had a protein content of 5.11% (Table 2). These results align with Zhong et al. (2007), who stated that processed sandfish have a higher protein content than fresh sandfish. The drying significantly reduces water content but increases the percentage of other components, such as proteins. The high protein content of dried sandfish confirms that sea cucumbers are rich-protein animals, wherein the protein has a complete composition of amino acids, both essential and non-essential (Chen et al., 2010).

The fat content of dried *H. scabra* was 0.62 – 1.15%, which agrees with the study of Ibrahim et al. (2015), which reported 1.03% of fat content in the dried *H. scabra*. On the contrary, the control had a very low-fat content, i.e., 0.03%, because water dominated the composition of fresh sandfish. Lastly, the difference method shows that the carbohydrate content of dried *H. scabra* was between 7.63% and 15.97%. These values are in line with several numbers reported by others. Nursid et al. (2022) reported that dried *H. atra* contains 6.9 – 9.7% carbohydrates, whereas Karnila

et al. (2011) reported the carbohydrate content of dried *H. scabra* was 12.54%.

Saponin Content

Saponin, a major secondary metabolite in sea cucumbers, exhibits various biological activities and therapeutic effects (Sottorff et al., 2013). The saponin content of sandfish in this study is presented in Table 3.

Table 3 shows that boiled sandfish have higher saponin content than unboiled sandfish ($p<0.05$), but drying techniques did not affect the saponin content differently ($p>0.05$). The highest saponin content of all treatments was in the boiling and oven-drying treatment (N1P2), i.e., 7.418 mg quillaja/10mg extract. In contrast to dried sandfish, the fresh sandfish had much lower saponin content ($p<0.05$). The saponin content of sandfish without boiling was lower than boiled ones. Boiling facilitates the releasing of limestone from the skin of sea cucumbers, resulting in cleaner sea cucumbers than without boiling (Purcell, 2014). Clear skin of sea cucumbers would not leave hard limescale on the skin of dried sea cucumbers. As a result, the saponin extraction process was more accessible, and a higher saponin content was obtained. Akerina (2019) stated that processing processes, such as boiling, did not affect the presence of saponin in sea cucumbers. This may indicate that high-temperature treatments do not easily damage saponin.

Saponin from various types of sea cucumbers has been studied, especially those that relate to the anticancer activity of the saponin. A saponin from *H. scabra* with anticancer properties is defined as echinoside A. This compound induces apoptosis in HepG2 cells (liver cancer cells) (Wang et al., 2014). Furthermore, two saponin compounds with anticancer properties have been isolated from sandfish: Holothurin A and Holothurin B (Caulier et al., 2011). Other saponins, identified as scabraside A and B also have intense anticancer activity against several cancer cells (Han et al., 2009). The strong anticancer properties of saponins are related to their ability to damage the permeability of cancer cell membranes (Kalinin et al., 2008). Nursid et al. (2019) reported the cytotoxicity of 15 sea cucumber extracts from Tomini Bay, Sulawesi, against T47D cells. Therefore, further research needs to be done on whether saponin is the only agent responsible for its anticancer properties.

Table 3. The saponin content of sandfish was dried using different techniques.

Treatment	Saponin Content (mg quillaja/10 mg extract)
Fresh	2.646±0.077[a]
N1P1	7.050±0.213[b]
N1P2	7.418±0.243[b]
N2P1	5.584±0.235[c]
N2P2	5.491± 0.745[c]

Note: N1P1 (boiled, sun drying), N1P2 (boiled, oven drying), N2P1 (unboiled, sun drying), N2P2 (unboiled, oven drying). Treatments that do not share the same letters in the same column are significantly different by ANOVA ($p<0.05$).

Table 4. The collagen content of sandfish was dried using different techniques.

Treatment	Collagen content (mg collagen/ 30mg sandfish)
Fresh	4.248 ± 0.904[a]
N1P1	4.678 ± 0.986[a]
N1P2	4.207 ± 0.618[a]
N2P1	4.789 ± 0.581[a]
N2P2	4.667 ± 0.055[a]

Note: N1P1 (boiled, sun drying), N1P2 (boiled, oven drying), N2P1 (unboiled, sun drying), N2P2 (unboiled, oven drying). The same lowercase letters indicate no significant differences between the treatments.

Collagen Content

The body of a sea cucumber mainly comprises collagen protein (Siahaan et al., 2017; Siddiqui et al., 2013). Park et al. (2012) reported that the collagen composition of the whole body and body wall of *H. scabra* is 15.07 g/100g and 18.38 g/100g dry weight, respectively. Therefore, collagen from sea cucumbers is an attractive alternative for use as an ingredient in cosmetic products. The collagen content of sandfish dried using different techniques is presented in Table 4.

Sun and oven drying, both with boiling and without boiling, had no significant effect (p>0.05) on the collagen content of *H. scabra*. The collagen content was 4.207– 4.789 mg collagen/30 mg sandfish (Table 4). The collagen contents of *H. scabra* presented in Table 4 are higher than the collagen content reported by Dewi et al. (2023) on sea cucumbers *B. marmorata*, *H. atra* and *H. leucospilota*. The three sea cucumbers had collagen content of 0.60, 0.52, and 0,51 mg collagen/mg sea cucumber, respectively. The collagen content in sea cucumbers depends on the type of sea cucumbers, e.g., *S. hermanii* contained 0.66% collagen (Safithri et al., 2018), and *S. variegatus* contained 16.4% collagen (Fawzya et al., 2016). Some types of sea cucumbers have a higher content than other collagen sources. For example, the collagen of *H. cinerascens* is higher than that of pig, i.e., 72.2% compared to 64.7%, respectively, which was even higher than the collagen content of tilapia skin (67.33%). The collagen of sea cucumbers is mainly studied for its functionality as hydrolytic bioactive peptides, which can be used to repair damaged tissue (Park et al., 2012), antitumor (Zhou et al., 2012b), and antioxidant (Zhou et al., 2012a).

FTIR Profiles

Fourier Transform Infrared (FTIR) analysis shows spectra that indicate functional groups or metabolite profiles of dried and fresh sandfish extracts. The functional groups of the extract from dried sandfish obtained from sun and oven drying were similar, shown as red, green, blue, and orange lines in Figure 1. The extract of fresh sandfish (black line), however, shows slightly different profiles from the dried sandfish. In all treatments, FTIR spectra show strong absorption at wave numbers 3000-3500 cm^{-1}, which were carbonyl (C=O) and hydroxyl (OH) groups (Fig 1). A wave number of 1700 cm-1 also characterized the carbonyl group. Wave numbers 2800 – 2900 cm^{-1} indicate stretching vibration in the methylene group (CH_2). The strong absorption at 1600 cm^{-1} indicates the presence of an olefinic group (alkene) in the sandfish extract. The strong absorption at 1400 cm^{-1} in the extract of fresh sandfish showed aromatic groups, possibly from the aglycone groups of saponin. The strong intensity at 1100 cm^{-1} in the extract of fresh sandfish indicates C-N stretching amine. A band at 600 cm^{-1} in the extract of fresh sandfish may indicate hydrogen bending in the hydroxyl group. The differences in FTIR spectral intensity between the extract from fresh sandfish and dried sandfish were at 3400 cm^{-1}, 1600 cm^{-1}, 1400 cm^{-1} and 600 cm^{-1}. These indicate that the moisture content in the extract of fresh sandfish was higher than dried sandfish. Those differences could also indicate changes in the

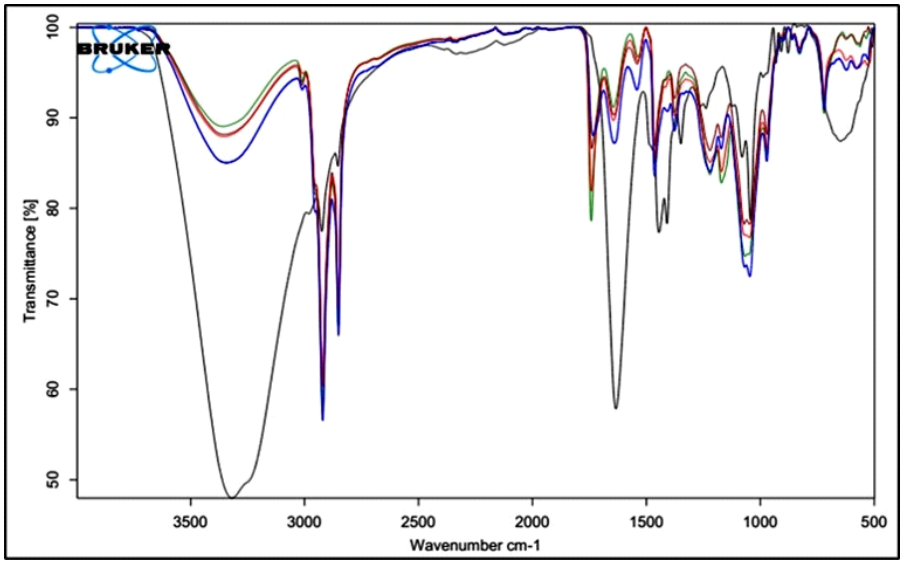

Figure 1. FTIR spectra analysis of sandfish dried using different techniques. Note: Red (N1P1, boiled, sun drying); green (N1P2, boiled, oven drying); blue (N2P1, unboiled, sun drying boiling); orange (N2P2, unboiled, oven drying); black (fresh sandfish).

composition of compounds containing amides. The FTIR characteristic peaks of amide I, amide II, and amide III bands are marked as amide or peptide characteristic peaks (Ji et al., 2020). Changes in the composition of amide-containing compounds were seen in dried sandfish. The strong intensity in fresh sandfish at 1600-1800 (amide I) was not found in dried sandfish. Likewise, at an intensity of 1470-1570 (amide II). According to Soltani (2014), functional groups that indicate the presence of saponins in sea cucumbers were detected at 2369 cm^{-1} (carboxylate), 2928 cm^{-1} (alkyl groups; C-H), and 1000–1300 cm^{-1} (ether; C-O and ester; –C=O). Oligosaccharide bonds with sapogenin (C-O-C) were detected in the spectrum at 1054-1261 cm^{-1}.

The chemical compositions in the extract of sandfish are complex. FTIR spectra might be helpful to describe the chemical characteristics of the extract (Tarapoulouzi et al., 2020), especially its essential functional groups. The spectra are also useful to follow changes in those functional groups by relating the band position and intensity of the spectra (Burhan et al., 2020). However, further analytical tools are required to describe better the differences in the chemical profiles of sea cucumber extracts and quantify them, e.g., by Liquid chromatography-mass spectrophotometry (LC-MS) or nuclear magnetic resonance (NMR).

Conclusion

The proximate composition, collagen content and FTIR profiles of sandfish (*H. scabra*) dried in the sun and oven, boiled or unboiled, did not show significant differences. However, the saponin content in boiled sandfish extract differed significantly from the saponin content in the unboiled sandfish extract. Boiling facilitates the release of lime content in the skin of sandfish, resulting in cleaner sandfish than unboiled sandfish. As a result, the saponin extraction process was more accessible, and a higher saponin content was obtained. These results also indicated that the bioactive compounds of sandfish, i.e., saponin and collagen, are heat stable. The results of this research imply that sandfish can be preserved in dried form either by sun or oven drying without a significant impact on its physicochemical composition. However, applying boiling or un-boiling as pre-treatment may partially impact the composition.

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Supplementary Materials

Supplementary materials is not available for this article

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