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Chemical compositions and nutritional profiles of two edible tunicate species (*Halocynthia roretzi* and *Halocynthia aurantium*)

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ABSTRACT

As an abundant marine bioresource, tunicates could be exploited in the food industry. However, limited knowledge of their chemical composition and nutritional profiles prohibited further application. In this study, two common edible tunicate species, *Halocynthia roretzi* (HR) and *Halocynthia aurantium* (HA), were subjected to comprehensive composition analysis in terms of moisture, protein, lipids, cellulose, ash, amino acids, fatty acids, non-cellulose carbohydrates and minerals. Reddish HR was much bigger than purple HA with respect to body length and weight, and their moisture fell within 82.98 %–90.92 %. The non-edible outer shell part (OS) and edible internal organs part (IO) had a dry weight ratio of around 3:2 for both two species. Generally, for both HR and HA, IO was more abundant in protein and lipids. In contrast, OS had much higher cellulose contents, confirming the better suitability of IO as a nutritional seafood. IO was richer in essential amino acids and unsaturated fatty acids, while OS had more abundant saturated fatty acids. The detected non-cellulose monosugars ranged from 0.47 % to 1.18 % and indicated the presence of some sulfated glycans. IO of HR had higher contents of essential minerals, such as Cu, Zn, and Fe, while IO of HA showed a higher K content. To sum up, this study identified the chemical composition and nutritional profile variations among different tunicate species and various dissected parts, guiding the development of specific strategies to exploit tunicates for proper food applications.

1. Introduction

Tunicates have a large biomass volume living in the ocean worldwide. They are prone to settle on solid substances. They are regarded as major aquatic invasive species, which could compete with shellfish to occupy the surface and reduce aquaculture production, thus leading to deleterious problems in marine ecosystems and food chains for human beings [1]. There are more than 2300 tunicate species worldwide. Due to higher growth rate, proliferation capacity, and survival abilities compared with sponges, shellfish

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and seaweeds, they have become a significant fouling animal species in the sea [2]. Many available studies have focused on the spreading and prohibiting of tunicates, expecting to guarantee aquaculture production [3]. Instead of disposing of the tunicates in the ocean, they show potential as valuable and abundant marine bioresources, which can be exploited for food, animal feed, material, and energy production [4,5]. Some edible tunicate species, such as *Halocynthia roretzi* (HR) and *Halocynthia aurantium* (HA), have been cultivated and consumed as delicious food in many countries [6].

In Asia, Chile, and some Mediterranean countries, many edible tunicate species are commonly available as fresh or dried products at seafood markets. The edible tunicate species can be eaten in various forms [6]. *Pyura chilensis,* locally called piure in Chile, is consumed domestically and exported to other countries, such as Sweden and Japan [7]. HA is usually called "sea peach" or "ice floe tunicate" (akaboya in Japan). It is dominantly cultivated in Japan, but no production data has been reported [8]. Another edible tunicate species, HR, commonly called "sea pineapple", was first farmed in Korea in 1982 [6]. Now, HR are cultivated on long-line systems in shallow subtidal areas in Korea and Japan. The production of HR was 6994 tons in 1991 and increased to 31,353 tons in 2016 [9]. *Styela plicata* is consumed fresh in both Korea and some Mediterranean countries. *Microcosmus hartmeyeri* (harutoboya in Japan) is eaten in Japan. *Microcosmus sabatieri* and *Microcosmus vulgaris* are consumed as famous recipes in France, Italy, and Greece. Historically, the Maoris in New Zealand consumed *Pyura pachydermatina*, which used to be a food source for aboriginal people living around Botany Bay, Australia [10]. Among all the mentioned edible tunicate species, the most common ones are HR and HA.

Chemical composition analysis is always the prerequisite to exploring food applications. Anatomically, all tunicates could be divided into two main parts, i.e., the outer shell and the internal organs. The outer shell is an external supportive tissue to hold the body shape and help the animals filter the sea waters. In addition, they can also help prohibit the prey from attacking [11]. The outer shell contains ~60 % cellulose, and another ~27 % by dry weight are nitrogen-containing organic ingredients [12]. The elemental composition of *Salpa thompsoni* has been determined, in which moisture is 93.6 % (aggregate form) and 92.3 % (solitary form). Ash content is as high as 44 % of the dry weight. Carbon and nitrogen contain 17–22 % and 3–5% of the dry weight [13]. It can be concluded that different tunicate species and fractions have different chemical compositions [14].

The outer shell and internal organs of tunicate also show different chemical compositions. Zhao et al. [15] analyzed the chemical compositions of three common tunicate species, *Ciona intestinalis*, *Styela plicata,* and *Ascidia* sp. The internal organs showed higher protein and lipids contents than the outer shell, while the latter ones are more abundant in cellulose. Of different species, *Ciona intestinalis* internal organs have the highest protein content of 69.32 % but the lowest cellulose content of 5.59 %. The cellulose content of *Styela plicata* outer shell is the highest, 57.67 %, and its lipid content is the lowest at 0.35 %. In addition to protein and lipids, since some tunicate species, such as *Ciona intestinalis*, has a unique capacity to accumulate iron (Fe) from the environment, it can be used as a useful indicator to reflect the environmental pollution for Fe. It also contains many different amounts of trace minerals, especially zinc (Zn), magnesium (Mg), vanadium (V), etc., which are also crucial in maintaining the healthy bodies of human beings [16].

The chemical contents of HR were previously analyzed [17], though the available data were limited and incomprehensive. HR has moisture at 77.5 %, crude protein at 11.3 %, crude lipid at 1.1 %, ash at 2.5 %, and glycogen at 6.6 %. 83 % of the extracted lipids are neutral lipids, while 17 % are phospholipids. Aspartate (Asp), glutamate (Glu), and Lysine (Lys) are the major amino acids with a content of 11425.4 mg%. 14:0, 16:0, 16:1-ω7, 18:1-ω7, 18:4-ω3, 20:5-ω3 and 22:6-ω3 are major fatty acids, and the content of ω3 polyunsaturated fatty acids is 39 %. The inorganic ingredients are mainly Na^+, K^+, Cl^-, and PO_4^{3-}. Zhao and his co-researchers also determined the principal chemical composition of HR [18]. They have a body size of 12.7 ± 4.5 cm (length) × 6.2 ± 2.6 cm (width) and an average body weight of 70.4 ± 16.7 g. 11.55 % ash, 38.08 % protein, 0.28 % lipids, and 46.52 % carbohydrate have been found in HR. In addition, HR is also rich in many bioactive compounds. For example, abundant carotenoids were present in HR, 47.87 mg/100 g for outer shell and 2.35 mg/100 g for internal organs [19]. For the outer shell, the major carotenoids included alloxanthin (31.3 %), halocynthiaxanthin (15.5 %), diatoxanthin (11.9 %), diadinochrome (11.6 %), mytiloxanthin (10.8 %) and astaxanthin (7.8 %). These carotenoids have shown anti-inflammatory, anti-angiogenesis, and anti-obesity effects [20].

Although HR and HA are consumed as seafood worldwide, very limited studies have been focused on the chemical composition and nutritional profile analysis of HR and HA. In addition, the available data largely concentrated on lipids, fatty acids and certain bioactive compounds, and the understanding on their chemical composition and nutritional profile is incomprehensive. Moreover, to our best knowledge, no study has been performed to investigate the differences of chemical composition and nutritional profile between these two different species, so that insufficient data on their safety as seafoods is available. Therefore, it is necessary further to analyze the chemical contents and nutritional profiles of these representative tunicate species, thus guiding their application exploration as healthy and safe seafood.

2. Materials and methods

2.1. Preparation of Tunicate sample

About 20 kg HR harvested in March 2023 was purchased from the Xunshan Fishery Company of Rongcheng, Shandong Province, China. HA was bought from a local fisherman who collected them in March 2023 from the Port of Xiamen, Xiamen Island, Fujian Province, China. Both HR and HA were intended to be sold as food in local markets, and they were received as dead in our lab. Based on the abovementioned information, this study had been exempted from ethics review by the Research Ethics Committee, Research Management Centre at Universiti Teknologi MARA, Malaysia (Reference number: REC/08/2023(PG/EX/42), see Fig. S3). All these tunicate samples were first cleaned thoroughly to eliminate the residual contaminants from the sea. Then, the animals were separated into the outer shell (OS) and internal organs (IO) (Fig. 1).

2.2. Freeze-drying of tunicate samples

The tunicate samples were dried in a freeze-dryer (Savant VLP-200, New York, USA) at −50 °C for three days. After freeze-drying, the samples were stored in a desiccator until further analysis.

2.3. Principle chemical contents analysis

2.3.1. Moisture content determination (AOAC, 1999)

The empty dish and lid were dried in the oven at 105 °C for 3 h and were transferred to a desiccator to cool. The dish was weighed without lid. About 3 g of the sample was loaded in the dish and dried at 105 °C for 3 h. The samples were cooled down in a desiccator. The dish and dried sample were reweighed to determine the moisture using the formula below:

$$Moisture\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1 is the weight of the sample before drying, and W_2 is the weight of the sample after drying.

2.3.2. Protein content analysis

The dried tunicate samples were hydrolyzed by mixing with 6 M HCl, heated at 110 °C for one day. The hydrolyzed product was subjected to a Total Nitrogen Module instrument (TNM-1 module, Shimadzu Scientific Instruments, Columbia, MD, USA) to determine the nitrogen content. Before measurement, the nitrogen analyzer was calibrated using 1, 5, 20, 50, and 100 ppm KNO_3 standard solutions. The determined nitrogen content was multiplied by 6.25 to calculate the "crude protein" content [21].

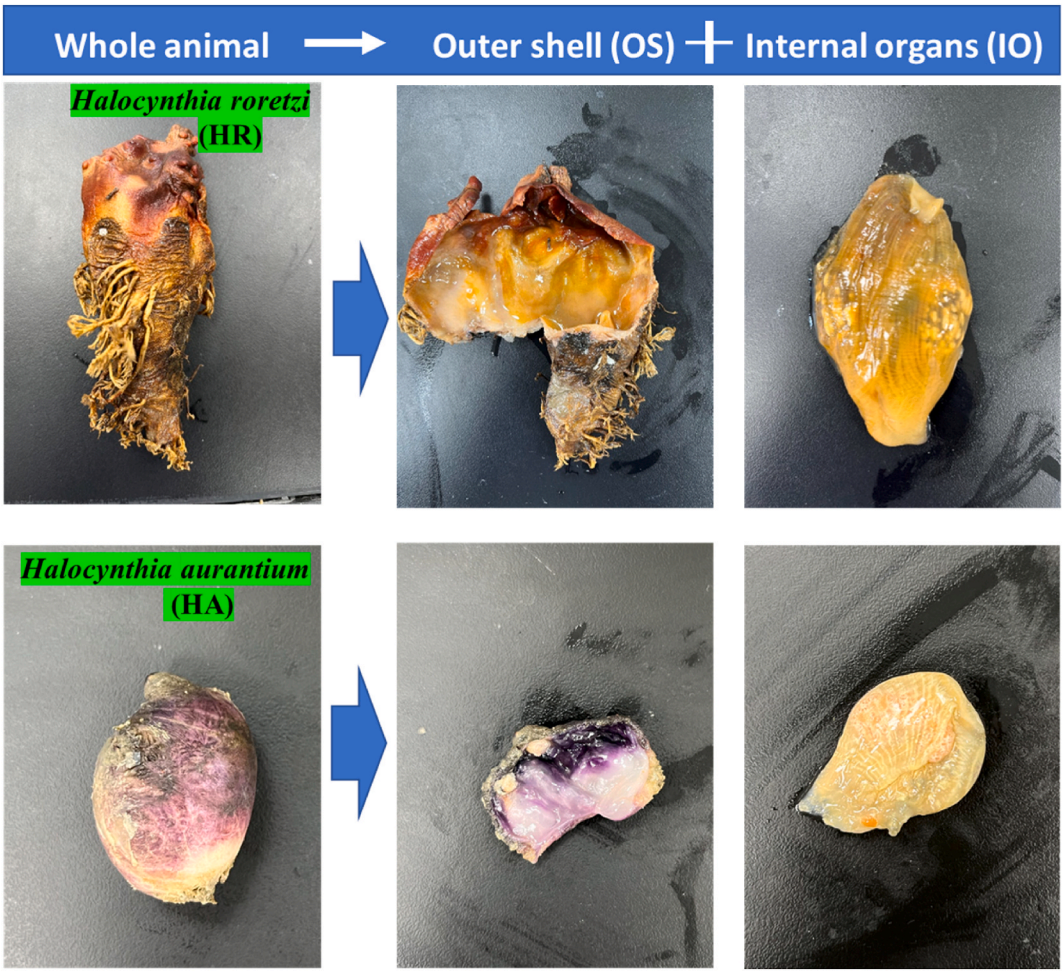

Fig. 1. The appearance of HR and HA and their corresponding OSs and IOs.

2.3.3. Lipid content determination

The dried tunicate samples were mixed with $CHCl_3$-methanol solution, and the mixture was extracted for four days to diffuse lipids completely. The lipids were collected by a vacuum evaporator (Buchi Labortechnik AG, Postfach, Switzerland) [22]. The lipids fractions were recovered using a rotary evaporator and weighed to calculate the gravimetric content.

2.3.4. Cellulose content analysis

The defatted samples were immersed in acetic-nitric reagents containing acetic acid, water, and nitric acid in a ratio of 8:2:1, which was subjected to heating in a water bath at 100 °C for 30 min to achieve the complete removal of non-cellulose components [23]. After the acid hydrolysis, this residue was collected and thoroughly washed several times to remove the excess acids. Then, the purified cellulose was freeze-dried and weighed to calculate the cellulose content.

2.3.5. Ash determination

The crucible was weighed and labeled as T, and the tunicate sample placed in the crucible was weighed and labeled as W. The tunicate sample was heated at > 600 °C for 16 h. After that, the crucible containing the ash inside was weighed and marked as R. Then the ash content was calculated by following the equation below.

$$\textit{Ash content}\ (\%) = \frac{R - T}{W - T} \times 100$$

2.4. Comprehensive chemical compositions analysis

2.4.1. Amino acids analysis

A previously reported method was used to determine the amino acid compositions of tunicate samples [24]. The tunicate samples were hydrolyzed to free amino acids, which were then analyzed by cation-exchange chromatography (1100 series HPLC system, Agilent, Waldbronn, Germany) on sulfonated polystyrene resins. The contents of Asp, threonine (Thr), serine (Ser), Glu, glycine (Gly), alanine (Ala), cysteine (Cys), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), Lys, NH_3, histidine (His) and arginine (Arg) were determined.

2.4.2. Fatty acid compositions

A ~20 mg dried sample was weighed; then, toluene (0.2 mL), methanol (1.5 mL), and an 8 % HCl solution in methanol (0.3 mL) were added sequentially to the sample. After sealing the ampoule, the mixture was heated to 100 °C for 1 h. Then, 1 mL of saturated sodium chloride solution and 1 mL of hexane were added, and the mixture was shaken for 1 min, followed by phase separation after allowing the solution to stand. The supernatant containing the fatty acid methyl esters (FAMEs) was used for GC-MS analysis. Tetracosane was used as an internal standard for calibration and quantification. A Hewlett-Packard 6890 gas chromatograph, equipped with a DB-5MS column (30 m length, 0.25 mm inner diameter, and 0.32 μm film thickness) (Wilmington, DE, USA), was used to separate the FAMEs. Helium was used as the carrier gas at a flow rate of 1 mL/min, incorporating a head pressure of 100 kPa and a 30:1 split ratio. Both the injector and detector temperatures were maintained at 250 °C. The temperature program was 60 °C (kept for 2 min) followed by 10 °C/min up to 200 °C (held for 2 min) and 5 °C/min up to 240 °C (kept for 7 min). Averages of triplicate injections were reported.

2.4.3. Monosaccharide analysis

Monosugar compositions of tunicate samples were determined using a Dionex HPAEC-PAD ionic chromatography (IC) system (Dionex ICS-3000, Dionex Spa, San Donato Milanese, Italy). Firstly, the samples were hydrolyzed by immersing the milled sample in 72 % H_2SO_4 and then diluted to 3 % H_2SO_4 until it was autoclaved at 120 °C for 60 min (cf. TAPPI Test Method T 249). The obtained hydrolysates were injected into the IC for monosugar analysis.

2.4.4. Mineral analysis

The dried sample was digested with concentrated HNO_3 and 30 % H_2O_2. Nitric acid was distilled with sub-boiling in a quartz apparatus. Digestion was performed in closed vessels in a microwave oven (CEM Mars Xpress, CEM Corporation, Matthews, NC, USA) at 180 °C. After that, the digests were diluted with Milli-Q water. An analysis was performed with ICP-MS (Thermo X series II, Thermo Fisher Scientific, Rochester, NY, USA), which was equipped with a collision cell. A collision gas (7 % H_2 in He) was used for V, chromium (Cr), Fe, nickel (Ni), arsenic (As), and selenium (Se) to reduce interferences and high background levels. Scandium (Sc), rhodium (Rh), and rhenium (Re) were used as internal standards.

2.5. Statistical analysis

All experiments were conducted in triplicate and repeated at least twice unless otherwise stated. SPSS software was applied to determine significance differences ($p < 0.05$) based on Analysis of variance (ANOVA) and Duncan's multiple ranges.

3. Results and discussions

3.1. Appearance difference between HR and HA

HR was cultivated in Rongcheng City, Shandong Province, and the farm was in the Yellow Sea. As shown in Fig. 1 and Table 1, HR was reddish with a body length of 9.91 ± 1.11 cm and a body weight of 111.72 ± 26.13 g. HA was collected from Xiamen City, Fujian Province. The collection area belonged to the East China Sea. Apparently, HA was different from HR, as it had purple skin and a much smaller body size (length of 2.45 ± 0.36 cm and weight of 3.19 ± 0.89 g). It had been reported that the size of the tunicate *Pyrosoma atlanticum* ranged from 13 to 25 cm, corresponding with wet weight varying from 22 to 64 g [25]. However, another tunicate species, *Salpa thompsoni*, was very small, has a 3–12 cm length, and a wet weight of 0.11–5.46 g [13]. It could be seen that the size of the tunicate varied from species to species.

HR and HA could be separated into two parts by easily peeling the skin: outer shells (OS) and internal organs (IO). Though OSs of HR (red and yellow) and HA (purple and white) showed different colors, IOs of both HR and HA were yellowish. The obtained parts were subjected to freeze-drying, and the moisture of the samples was determined. As shown in Table 2, OS accounted for 64.68 % of the body weight of wet HR, while the remaining part was IO (35.32 %). This was similar to HA, whose OS occupied 70.09 % while IO accounted for only 29.91 %. So, this suggested that for both HR and HA, the weight of the outer shell was more than two times higher than that of internal organs. It was well-known that only the IO part of tunicate was edible, weighing around one-third of the whole animal. Therefore, exploring the non-edible OS part for other applications would be critical to using tunicate as seafood. After drying, the weight ratio of IO slightly increased to around 40 % while OS's decreased to 60 %. This change originated from the moisture difference between these parts, as shown in Table 2. For both HR and HA, the moisture of OS was higher than IO, which might be due to the direct contact of the OS with seawater. HA showed higher moisture than HR irrespective of outer shells and internal organs, possibly due to their different living environment, HR in the Yellow Sea and HA in the East China Sea. Zhao et al. dissected tunicate *Styela plicata*, *Ascidia* sp., and *Ciona intestinalis* into OSs and IOs, and the moisture was determined to be 88–94 % and 84–97 %, respectively [18]. This suggested that the moisture of tunicate parts were species- and part-dependent.

3.2. Principle chemical composition of HR and HA

All the obtained tunicate samples were subjected to chemical analysis, and their principal chemical composition is shown in Table 3. For HR, IO had a slightly higher protein content (48.41 %) than OS (42.12 %), indicating that the non-edible part even had relatively abundant protein. However, the OS of HA only had 27.40 % protein, significantly lower than 40.53 % for IO. OS of both HR and HA showed similar cellulose content, 31.05 % and 28.58 %, respectively, two times higher than those present in their corresponding IO parts. As reported by Zhao et al. (2014), OS functions as a supportive part to protect the tunicates from waves and predators and helped hold the body shape in the filter-feeding process, so the high cellulose content as a reinforcing component played a vital role in those functions [15]. Apart from cellulose, some non-cellulose carbohydrates were also observed, with a minimal content of 0.47 %–1.18 %. According to a previous study, many glycosaminoglycan (GAG) and GAG-like polysaccharides with anti-inflammatory activity were extracted and purified from various tunicate species, such as *Ascidiella aspersa* [26], *Ciona intestinalis,* and *Herdmania monus* [27]. The presence of specific non-cellulose carbohydrates in HR and HA might also suggest the existence of these bioactive polysaccharides. As shown in Table 3, HR and HA were also similar in lipid content, 18.42 % and 19.43 % for IO, 2.71 % and 3.12 % for OS, respectively. The IO was the edible part of the tunicate, which contained digestive organs, reproductive organs, and a branchial basket for filter-feeding, so the high lipid content in IO was necessary to realize many life activities. Regarding the inorganic component, an ash content of 17.93 % was observed in the OS of HR, while IO had an ash content of 21.72 %. HA generally showed higher ash contents than HR, 29.14 % for OS and 35.18 % for IO. As shown in Table 2, higher moisture was observed for HA, which meant that more seawater was trapped in the animal body, and the high salinity of this seawater should be the reason for the difference in ash content.

Samuelsen et al. determined the proximate chemical composition of *Ciona intestinalis* and found that it contained 36.8 % protein, 24.1 % carbohydrate, 3.5 % lipids, and 35.5 % ash [5]. The observed protein content of 27.40–48.41 % agreed well, though the lipid content in IOs of HR (18.42 %) and HA (19.43 %) was significantly higher than that of *Ciona intestinalis* (3.5 %). HR had reported having crude protein 11.3 %, crude lipid 1.1 %, ash 2.5 %, and glycogen 6.6 % [17]. It was evident that the observed protein, lipids, and ash contents of HR in this study were much higher than their findings, which might be due to the different living environments of the HR used in these two studies.

Table 1
Body size of wet tunicates HR and HA[a].

	Body weight (g)	Body length (cm)
HR	111.72 ± 26.13	9.91 ± 1.11
HA	3.19 ± 0.89	2.45 ± 0.36

[a] HR, *Halocynthia roretzi* and HA, *Halocynthia aurantium*.

Table 2
Weight ratio and moisture of different parts of HR and HA[a].

	Part	Weight percentage (%, in wet)	Weight percentage (%, in dry)	Moisture (%)
HR[b]	OS	64.68 ± 1.02[a]	59.76 ± 0.56[a]	85.73 ± 2.34[b]
	IO	35.32 ± 0.90[b]	40.24 ± 1.13[b]	82.98 ± 1.05[c]
HA	OS	70.09 ± 0.76[a]	61.51 ± 1.09[a]	90.92 ± 1.98[a]
	IO	29.91 ± 2.17[b]	38.49 ± 0.45[b]	86.69 ± 0.73[b]

[a] Different superscripts in the same column indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

Table 3
Principle chemical composition of HR and HA[a].

		Protein (%)	Cellulose (%)	Non-cellulose carbohydrate (%)	Lipids (%)	Ash (%)
HR[b]	OS	42.12 ± 0.42[b]	31.05 ± 0.86[a]	1.18 ± 0.04[a]	2.71 ± 0.02[b]	17.93 ± 1.52[d]
	IO	48.41 ± 1.03[a]	14.78 ± 0.32[c]	0.47 ± 0.03[c]	18.42 ± 0.08[a]	21.72 ± 0.59[c]
HA	OS	27.40 ± 0.67[c]	28.58 ± 1.24[b]	0.90 ± 0.07[b]	3.12 ± 0.02[b]	29.14 ± 0.87[b]
	IO	40.53 ± 0.29[b]	14.25 ± 0.75[c]	0.92 ± 0.03[b]	19.43 ± 0.07[a]	35.18 ± 1.22[a]

[a] Different superscripts in the same column indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

3.3. Comprehensive chemical composition analysis of HR and HA

In order to get more detailed information regarding the nutrition and safety of these two common edible tunicates, comprehensive chemical composition analyses of HR and HA were performed in terms of amino acid profile, fatty acid composition, monosaccharide profile of non-cellulose carbohydrate, and mineral composition.

3.3.1. Amino acid distribution

As shown in Table 4, HR was more abundant in Val, Ile, Leu, and Phe than in HA. It should be noted that all these amino acids were essential amino acids (EAA), suggesting that HR as seafood was higher nutritional. The observed difference in amino acids of HR and HA might be possibly due to their food availability in the living environments. In fact, HR was cultured in a farm, in which the foods, mainly algae, were sufficiently provided to boost their optimal growth. However, HA was widely collected, and its growth was dependent to the available foods in the sea, which was less controllable than those in the farm. In HA, a higher content of NH_3 was observed, 56.05 % for OS and 21.97 % for IO. Among all parts of different tunicate species, IO of HR was unique, characterized by an extremely high content of 13.15 % Cys, 6.54 % Ile, 10.11 % Leu, and 10.50 % Lys, corresponding to the most abundant EEA of 48.56 %

Table 4
Amino acid distribution of HR and HA (%)[a].

	HR[b]		HA	
	OS	IO	OS	IO
Asp	4.03 ± 0.02[a]	0.69 ± 0.01[b]	4.31 ± 0.02[a]	1.52 ± 0.02[b]
Thr	6.57 ± 0.02[a]	1.76 ± 0.03[c]	1.18 ± 0.02[d]	3.50 ± 0.01[b]
Ser	4.15 ± 0.03[a]	0.26 ± 0.01[d]	1.14 ± 0.01[b]	0.90 ± 0.01[c]
Glu	17.60 ± 0.01[a]	9.58 ± 0.02[b]	18.21 ± 0.02[a]	7.47 ± 0.01[c]
Gly	15.06 ± 0.02[b]	3.64 ± 0.02[c]	3.33 ± 0.02[c]	26.21 ± 0.02[a]
Ala	8.67 ± 0.02[c]	11.00 ± 0.02[b]	5.12 ± 0.03[d]	15.37 ± 0.01[a]
Cys	0.98 ± 0.01[b]	13.15 ± 0.04[a]	1.01 ± 0.01[b]	1.78 ± 0.04[b]
Val	5.67 ± 0.02[b]	8.43 ± 0.01[a]	2.05 ± 0.02[d]	2.87 ± 0.03[c]
Met	0.75 ± 0.04[b]	1.17 ± 0.02[a]	0.89 ± 0.02[b]	1.04 ± 0.04[a]
Ile	4.45 ± 0.01[b]	6.54 ± 0.01[a]	1.09 ± 0.01[d]	2.43 ± 0.02[c]
Leu	6.85 ± 0.01[b]	10.11 ± 0.01[a]	1.06 ± 0.03[d]	3.82 ± 0.02[c]
Tyr	0.69 ± 0.02[d]	2.88 ± 0.02[b]	1.41 ± 0.02[c]	3.31 ± 0.01[a]
Phe	8.09 ± 0.03[a]	7.78 ± 0.02[a]	1.04 ± 0.02[b]	3.96 ± 0.02[b]
Lys	2.87 ± 0.04[b]	10.50 ± 0.02[a]	1.35 ± 0.01[d]	1.97 ± 0.03[c]
NH_3	9.76 ± 0.02[c]	9.45 ± 0.01[c]	56.05 ± 0.01[a]	21.97 ± 0.02[b]
His	1.48 ± 0.02[b]	2.27 ± 0.03[a]	0.27 ± 0.01[d]	1.11 ± 0.02[c]
Arg	2.34 ± 0.02[a]	0.79 ± 0.02[b]	0.50 ± 0.02[c]	0.77 ± 0.01[b]
EAA	36.73 ± 0.05[b]	48.56 ± 0.02[a]	8.93 ± 0.02[d]	20.69 ± 0.01[c]
SEAA	1.67 ± 0.01[d]	16.03 ± 0.02[a]	2.42 ± 0.01[c]	5.10 ± 0.01[b]
NEAA	61.60 ± 0.04[c]	35.41 ± 0.01[d]	88.65 ± 0.02[a]	74.21 ± 0.02[b]
EAA/NEAA ratio	0.60 ± 0.02[b]	1.37 ± 0.01[a]	0.10 ± 0.01[d]	0.28 ± 0.02[c]

[a] Different superscripts in the same row indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

compared with 36.73 % for OS of HR, 8.93 % for OS of HA and 20.69 % for IO of HA, respectively. In addition, its semi-essential amino acids (SEAA) also showed the highest content of 16.03 %, suggesting the better nutritional profile of IO of HR than other tunicate parts. Zhao et al. (2016) measured the amino acid composition of two non-edible tunicate species, *Ascidia* sp. and *Ciona intestinalis* [18]. It had been found that the IOs of these two species had 32.73 % and 45.57 % EAA, respectively, and the observed 48.56 % EAA for IO of HR indicated its better nutritional profile. However, the EAA content in IO of HA was only 20.69 % while the remaining parts were non-essential amino acids (NEAA), suggesting its low nutritional value. This was further confirmed by the calculated EAA/NEAA ratio, in which the value of 1.37 was found for the IO of HR, significantly higher than 0.10–0.60 for other parts. Although IO from both HR and HA were edible, our findings suggested that HR was considered more nutritional than HA regarding amino acid profile.

3.3.2. Fatty acid composition

GC-MS analyzed the fatty acid composition of tunicate parts, and the results are presented in Fig. S1 and Table 5. It had been found that OS had higher SFA contents than IO, 81.86 % vs. 34.05 % for HR and 69.61 % vs. 48.36 % for HA, respectively. C16:0 was the most abundant SFA for all samples, followed by C18:0 and C14:0. Short-chain SFA, such as C8:0, C10:0, and C12:0, were only observed in HA. Their absence in HR might suggest the different food systems for these two tunicate species distributed in different seas. IO of HR had the highest content of USFA (65.95 %), of which 16.94 % was MUFA while 49.01 % was PUFA. However, only 18.14 % USFA was found in the OS of HR. A similar difference was also found for HA. In addition, IO of HR was also characterized by the highest contents of ω3 FA (36.02 %) and ω6 FA (7.04 %), further suggesting the better lipids quality present in this part.

Culkin and Morris (1970) determined the fatty acid contents of two tunicate species, *Pyrosoma* and *Salpa cylindrical*. They found that these two tunicate species were rich in myristic acid (C14:0), 13.9 % and 12.6 %, respectively. However, the commonly found polyunsaturated acid C22:6 and the polyunsaturated C16 fatty acids in phytoplankton were not abundant [28]. This was consistent

Table 5
Fatty acid composition of HR and HA (%)[a].

		HR[b]		HA	
		OS	IO	OS	IO
Saturated FA	C8:0	n.d.	n.d.	0.32 ± 0.01	0.51 ± 0.02
	C10:0	n.d.	n.d.	0.47 ± 0.01	1.13 ± 0.01
	C12:0	n.d.	n.d.	2.98 ± 0.02	n.d.
	C13:0	n.d.	0.30 ± 0.02^{b}	1.20 ± 0.01^{a}	0.28 ± 0.02^{b}
	C14:0	2.95 ± 0.01^{c}	7.10 ± 0.03^{b}	9.83 ± 0.02^{a}	10.36 ± 0.02^{a}
	C15:0	0.66 ± 0.02^{d}	2.03 ± 0.01^{c}	3.13 ± 0.02^{a}	2.75 ± 0.02^{b}
	C16:0	44.08 ± 0.04^{a}	13.47 ± 0.02^{d}	31.31 ± 0.02^{b}	21.69 ± 0.01^{c}
	C17:0	0.75 ± 0.01^{d}	1.31 ± 0.01^{c}	2.67 ± 0.02^{a}	1.90 ± 0.01^{b}
	C18:0	31.92 ± 0.02^{a}	7.17 ± 0.01^{c}	13.64 ± 0.02^{b}	7.98 ± 0.01^{c}
	C19:0	0.78 ± 0.02^{b}	0.98 ± 0.02^{a}	0.80 ± 0.01^{b}	0.62 ± 0.03^{c}
	C20:0	0.72 ± 0.01^{c}	0.85 ± 0.02^{b}	1.16 ± 0.02^{a}	0.80 ± 0.01^{b}
	C21:0	n.d.	0.32 ± 0.03^{b}	0.46 ± 0.03^{a}	0.33 ± 0.02^{b}
	C22:0	n.d.	0.29 ± 0.01	0.98 ± 0.02	n.d.
	C23:0	n.d.	0.11 ± 0.02	0.24 ± 0.01	n.d.
	C24:0	n.d.	0.12 ± 0.01	0.42 ± 0.02	n.d.
Unsaturated FA	C14:1	n.d.	0.02 ± 0.00	n.d.	n.d.
	C16:1	1.46 ± 0.02^{d}	3.36 ± 0.02^{c}	7.09 ± 0.04^{a}	5.12 ± 0.01^{b}
	C16:2	n.d.	0.30 ± 0.01^{a}	0.24 ± 0.01^{b}	0.34 ± 0.02^{a}
	C17:1	n.d.	0.21 ± 0.01^{b}	0.66 ± 0.01^{a}	0.29 ± 0.02^{b}
	C18:3	n.d.	1.06 ± 0.02^{b}	0.82 ± 0.04^{c}	6.02 ± 0.01^{a}
	C18:4	n.d.	2.06 ± 0.03	n.d.	n.d.
	C18:2	1.11 ± 0.02^{c}	2.78 ± 0.02^{a}	2.29 ± 0.03^{b}	2.78 ± 0.01^{a}
	C18:1	3.92 ± 0.02^{c}	10.75 ± 0.04^{b}	13.27 ± 0.04^{a}	10.61 ± 0.02^{b}
	C20:4	2.71 ± 0.01^{c}	4.26 ± 0.01^{a}	1.40 ± 0.01^{d}	3.24 ± 0.02^{b}
	C20:5	2.61 ± 0.02^{c}	18.51 ± 0.03^{a}	2.16 ± 0.01^{c}	11.48 ± 0.01^{b}
	C20:3	n.d.	0.49 ± 0.01	n.d.	n.d.
	C20:2	n.d.	0.34 ± 0.01	n.d.	n.d.
	C20:1	0.70 ± 0.01^{b}	1.92 ± 0.01^{a}	0.61 ± 0.02^{b}	0.65 ± 0.01^{b}
	C21:5	n.d.	0.95 ± 0.02	n.d.	n.d.
	C22:5	n.d.	1.81 ± 0.02	n.d.	10.22 ± 0.05
	C22:6	1.56 ± 0.01^{b}	16.45 ± 0.05^{a}	1.85 ± 0.01^{b}	0.89 ± 0.02^{c}
	C22:1	4.07 ± 0.03	0.57 ± 0.01	n.d.	n.d.
	C24:1	n.d.	0.11 ± 0.01	n.d.	n.d.
SFA		81.86 ± 0.03^{a}	34.05 ± 0.02^{d}	69.61 ± 0.06^{b}	48.36 ± 0.03^{c}
MUFA		10.15 ± 0.04^{c}	16.94 ± 0.01^{b}	21.63 ± 0.01^{a}	16.66 ± 0.03^{b}
PUFA		7.99 ± 0.01^{c}	49.01 ± 0.02^{a}	8.76 ± 0.03^{c}	34.98 ± 0.02^{b}
USFA		18.14 ± 0.03^{d}	65.95 ± 0.04^{a}	30.39 ± 0.02^{c}	51.64 ± 0.02^{b}
ω3 FA		4.17 ± 0.02^{c}	36.02 ± 0.02^{a}	4.83 ± 0.01^{c}	18.40 ± 0.01^{b}
ω6 FA		3.82 ± 0.02^{c}	7.04 ± 0.01^{a}	3.69 ± 0.01^{c}	6.02 ± 0.02^{b}

[a] Different superscripts in the same row indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

with our findings that 2.95%–10.36 % C14:0 was detected in different parts of HR and HA, while no C22:6 was observed. Oh et al. (1997) determined the fatty acid composition of HR and found that 14:0, 16:0, 16:1-ω7, 18:1-ω7, 18:4-ω3, 20:5-ω3 and 22:6-ω3 were major fatty acids, and the content of ω3 PUFA was 39 % [17]. In this study, the determined ω3 PUFA content was 36.02 %, similar to previous studies.

3.3.3. Monosaccharide composition of non-cellulose carbohydrate

Though cellulose was the major carbohydrate in tunicate, certain non-cellulose carbohydrates were also present, which might play critical roles in many life activities. This study analyzed the monosaccharide composition of non-cellulose carbohydrates in tunicates (Fig. S2). As shown in Table 6, for both HR and HA, higher contents of ribose were found in OS, while IO was more abundant in glucuronic acid. Polysaccharides containing uronic acid were known to display significant antioxidative activity. Therefore, the high glucuronic acid content in IO of tunicates might possess such biological activity [29]. Other monosaccharides, such as mannose, galactose, and fucose, were also detectable in these tunicate samples. As reported previously, certain tunicate species could be a source of many non-cellulose carbohydrates, such as sulfated L-galactan from *Styela plicata* [30], sulfated mannose homopolysaccharide from *Didemnum mole* [31], and sulfated α-L-galactofucan from *Clavelina* sp. [32], the detected mannose, galactose, and fucose in HR and HA might indicate the presence of these sulfate polysaccharides in these tunicate species. Since these sulfate polysaccharides showed many biological properties, such as antioxidant, anti-inflammatory, anti-coagulant activity, and even anti-HIV properties [26,31,], these tunicate species should be further explored regarding these biological compounds for potential biomedical applications.

3.3.4. Mineral composition

Since some minerals might affect the safety of the tunicates as seafood, it was necessary to investigate the mineral composition of tunicates. As shown in Table 7, the total mineral content of OI was higher than OS, namely 63955.0 ppm vs. 49620.8 ppm and 99284.2 ppm vs. 87571.2 ppm for HR and HA, respectively. This was consistent with the ash contents of the samples. According to previous studies, tunicate had a strong accumulating capacity on specific metal ions, such as V [33]. V at 2.1–6.3 ppm was present as vanadium-binding proteins (VBPs) in HR, associated with antidiabetic effects due to the vanadium insulin-like activity [34]. In this study, the V content in IO of HR was 13 ppm, consistent with previous findings. However, V was not detected in OS, which also agreed well with earlier studies that V was only found in blood plasma, intestines, and muscles of HR. Compared with HR, HA had similar V contents, 12.9 ppm and 14.1 ppm for OS and IO, respectively. IO of HR had the highest content of Cu (103.5 ppm) and Zn (654.7 ppm) than other samples; since these were essential elements to humans, they might have health benefits while present in the seafood. Aluminum (Al) content was significantly higher in HA than in HR, namely 13948.1 ppm and 16847.3 ppm for the former, while 1662.5 ppm and 6626.9 ppm for the latter. In addition, lead (Pb), a toxic metal ion, was detected in HA with 10.1 ppm and 9.9 ppm for OS and IO, respectively. As mentioned in the Materials and Methods section, HR was cultured on a farm in Rongcheng, China, so the water quality was artificially controlled, thus avoiding the existence of toxic metal ions. However, HA was widely collected along the shores in Xiamen, China, and the presence of heavy metal ions should be related to the water pollution from the industry of the city. This confirmed the previous findings that tunicate species could be marine pollution indicators [16].

In previous studies, 0.5 ppm Pb was detected in *Microcosmus sabatieri*, which was considered a safe food source [35]. However, a much higher content of Pb (around 10 ppm) was found for HA, which was much higher than the maximum levels set by the European legislation (1.5 mg Pb kg^{-1}), indicating its high risk for food consumption. Zhao et al. (2016) determined the Al content in three tunicate species, and the concentrations were found to be 16354.30 ppm, 897.56 ppm, and 957.09 ppm for *Styela plicata*, *Ascidia* sp., and *Ciona intestinalis*, respectively [18]. It could be seen that HA had similar Al content to *Styela plicata* but was significantly higher than that of other species. Due to the adverse effects of Al on human health, such as Alzheimer's disease, dementia, hyperactivity, and learning disorders [36], our findings indicated that HA might not be suitable as a seafood.

3.4. Comparison of HR and HA with other seafoods

The Pacific oyster (*Crassostrea gigas*) was well accepted as a delicious and healthy seafood, which had been reported to contain protein (39.1–53.1 %), lipids (7.8–8.7 %), carbohydrate (21.6–38.9 %) and ash (4.0–12.1 %) [37]. In our study, the IO of both HR and

Table 6
Monosaccharide composition of non-cellulose carbohydrates in HR and HA (%)[a].

	HR[b]		HA	
	OS	IO	OS	IO
Mannose	0.02 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Ribose	0.70 ± 0.04[a]	0.25 ± 0.01[d]	0.56 ± 0.06[b]	0.32 ± 0.03[c]
Glucuronic acid	0.08 ± 0.02[b]	0.12 ± 0.02[a]	0.09 ± 0.01[b]	0.30 ± 0.02[c]
Galacturonic acid	0.01 ± 0.00[b]	0.03 ± 0.01[a]	0.02 ± 0.01[b]	0.01 ± 0.00[b]
Glucose	0.05 ± 0.01[b]	0.05 ± 0.02[b]	0.08 ± 0.02[b]	0.17 ± 0.03[a]
Galactose	0.21 ± 0.03	n.d.	0.10 ± 0.01	n.d.
Fucose	0.11 ± 0.03[a]	n.d.	0.04 ± 0.01[b]	0.11 ± 0.01[a]
Total content	1.18 ± 0.02[a]	0.47 ± 0.04[c]	0.90 ± 0.02[b]	0.92 ± 0.01[b]

[a] Different superscripts in the same row indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

Table 7
Mineral composition of HR and HA (ppm)[a].

		HR[b]		HA	
		OS	IO	OS	IO
Essential elements	V	n.d.	13.0 ± 1.2[a]	12.9 ± 0.8[a]	14.1 ± 0.4[a]
	Ni	8.0 ± 0.2[c]	n.d.	14.3 ± 0.1[b]	16.4 ± 0.2[a]
	Cr	13.8 ± 0.1[c]	27.0 ± 0.3[a]	27.1 ± 1.1[a]	18.7 ± 1.7[b]
	Cu	26.7 ± 0.9[b]	103.5 ± 1.6[a]	23.2 ± 1.8[b]	11.6 ± 0.4[c]
	Zn	56.9 ± 0.2[c]	654.7 ± 2.2[a]	48.0 ± 1.0[d]	95.1 ± 0.5[b]
	Sn	108.2 ± 1.5[a]	52.6 ± 1.0[c]	52.6 ± 0.1[c]	86.8 ± 0.1[b]
	Mn	770.7 ± 2.7[a]	138.9 ± 0.4[d]	517.8 ± 1.5[b]	399.2 ± 0.7[c]
	Fe	1386.0 ± 19.1[c]	5938.9 ± 10.5[b]	6007.0 ± 8.9[b]	6988.0 ± 23.2[a]
Other elementsa	K	2609.3 ± 101.2[d]	9223.7 ± 45.3[b]	7629.1 ± 10.4[c]	10309.4 ± 32.8[a]
	Mg	4122.3 ± 21.9[c]	2996.9 ± 10.8[d]	6233.9 ± 4.9[b]	7180.1 ± 50.2[a]
	Ca	4773.2 ± 10.3[a]	3836.9 ± 3.7[b]	3636.5 ± 28.6[c]	2353.2 ± 19.2[d]
	B	54.1 ± 1.2[a]	13.7 ± 0.9[d]	42.6 ± 1.8[b]	29.1 ± 1.1[c]
	Ti	57.9 ± 0.5[d]	262.9 ± 1.7[c]	521.3 ± 0.2[a]	302.4 ± 2.4[b]
	Sr	70.6 ± 5.6[a]	35.2 ± 0.3[c]	53.8 ± 3.7[b]	48.7 ± 2.5[b]
	S	9265.4 ± 32.7[c]	8866.6 ± 10.2[d]	15782.4 ± 5.7[a]	9918.7 ± 29.4[b]
	Na	21297.0 ± 78.0[c]	19556.6 ± 10.6[d]	30032.3 ± 114.3[b]	36149.5 ± 90.6[a]
	P	854.8 ± 0.6[d]	4943.2 ± 11.9[b]	1969.8 ± 18.1[c]	6413.2 ± 10.3[a]
	Al	1662.5 ± 89.2[d]	6626.9 ± 45.8[c]	13948.1 ± 60.6[b]	16847.3 ± 145.2
	As	n.d.	18.9 ± 0.6[a]	9.9 ± 1.8[c]	14.2 ± 2.1[b]
	Si	2430.6 ± 12.3[a]	567.4 ± 15.7[d]	932.2 ± 7.4[c]	1988.7 ± 5.3[b]
	Li	n.d.	9.4 ± 0.1	n.d.	12.8 ± 0.1
	Zr	n.d.	9.8 ± 1.2[a]	9.5 ± 0.5[a]	10.3 ± 0.3[a]
	Pb	n.d.	n.d.	10.1 ± 0.7	9.9 ± 0.2
	Ce	n.d.	n.d.	13.0 ± 1.6	13.5 ± 0.9
	Au	9.1 ± 0.6	n.d.	n.d.	n.d.
	La	15.3 ± 2.7	11.9 ± 0.5	n.d.	n.d.
	Ba	19.1 ± 1.2[c]	46.1 ± 4.3[b]	43.7 ± 1.8[b]	53.2 ± 2.3[a]
Total content		49620.8 ± 109.6[d]	63955.0 ± 67.2[c]	87571.2 ± 165.4[b]	99284.2 ± 56.9[a]

[a] Different superscripts in the same row indicate significant differences at $p < 0.05$.
[b] HR, *Halocynthia roretzi*, HA, *Halocynthia aurantium*, OS, outer shells and IO, internal organs.

HA contained 40–48 % protein, 18–19 % lipids, around 15 % carbohydrates and 22–35 % ash. It could be seen that the protein and carbohydrate contents of tunicates were similar to the Pacific oyster. In contrast, the lipids content was much higher than Pacific oyster, suggesting that tunicates had comparable nutritional profiles to Pacific oyster. In addition, we found that IO of HR was characterized by high contents of PUFAs (49.01 %), ω3 FA (16.02 %), and ω6 FA (7.04 %). A previous study investigated the fatty acid composition in the edible meat of twenty-nine species of wild and cultured freshwater and marine fish and shrimps. It was found that the levels of total PUFAs varied from 16.1 % in white Chinese croaker to 41.1 % in melon seed, while the levels of ω3 FA and ω6 FA were within 14.9–35.2 % and 1.4–5.9 %, respectively [38]. The fatty acid profile of tunicates was comparable to or even better than that of commonly consumed fish, shrimp, and other seafood.

4. Conclusions

IO was generally more abundant in protein and lipids, while OS had much higher cellulose contents, suggesting IO's better suitability as a nutritional seafood. However, a significant amount of protein was also present in OS, indicating their potential exploration as protein sources in animal feed. IO of HS showed the exceptionally highest EAA content, confirming the better amino acid quality than other tunicate parts. OS was much richer in saturated fatty acids (SFA), demonstrating that the SFA-containing lipids with cellulose built up the protective outer shell to prevent predators. IO had higher contents of unsaturated fatty acids (USFA), indicating they were more involved in life activities. In addition, the detection of non-cellulose carbohydrates might reveal the presence of some simulated glycans with biological activities. HR was much richer in essential minerals, such as Na, Fe, Ca, and K, while significantly high toxic metal ions, Al and Pb, were detected in HA. The findings in this study suggested that HR was more suitable as seafood than HA in terms of rich nutrients, high-quality amino acids, fatty acids, more essential minerals, and less toxic metal ions.

Data availability statement

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Pingping Gao: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Heng Yen Khong:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Agustono Wibowo: Writing – review & editing, Supervision. **Yixiang Zhen:** Data curation. **Chengcheng Peng:** Data curation. **Wenhua Miao:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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