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Composition and content of fatty acid, lipid classes of two octopus species: *Amphioctopus neglectus* and *Cistopus indicus* from Ha Long bay, Quang Ninh province, Vietnam

Dinh Thi Ha[1], Doan Lan Phuong[2], Pham Van Chien[1], Nguyen Van Quan[1], Nguyen Duc The[1,*]

[1]*Institute of Marine Environment and Resources, VAST, Vietnam*
[2]*Institute of Natural Products Chemistry, VAST, Vietnam*

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ABSTRACT

The fatty acid compositions of the lipids of two octopus species (*Amphiotopus neglectus* and *Cistopus indicus*) were investigated. The composition and content of fatty acids of two octopus samples were analyzed using a GC-MS. The percentages of the lipid classes in total lipid were determined based on the image analysis program Sorbfil TLC Videodensitometer DV, Krasnodar, Russia. The highest content of docosahexaenoic acid (DHA) (from 20.65% to 34.56% of total fatty acids) in the lipids of both species was revealed, followed by the content of eicosapentaenoic acid (EPA), palmitic acid, and arachidonic acid (AA) in the ranges (12.76–21.54%; 12.23–14.96%; and 8.99–17.26% of total fatty acids, respectively). The content of unsaturated fatty acids did differ between males and females of the two species studied. However, these differences are few and can be explained by the timing of their breeding seasons. The fatty acid composition of the fats from both investigated octopus species permits their use as food with nutritional value, and the production of bioactive additives.

Keywords: Fatty acid, lipid class, total lipid, octopus, *Amphiotopus neglectus*, *Cistopus indicus.*

[*]Corresponding author at: Institute of Marine Environment and Resources, 246 Da Nang, Ngo Quyen, Hai Phong, Vietnam. *E-mail addresses:* ductheimer@gmail.com

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INTRODUCTION

The cephalopods represent an essential economic seafood for human consumption. Due to their nutritional and market value, cephalopod aquaculture has also increased during the past few years [1]. Octopus, a representative of the cephalopod class, is one of the seafood species with abundant reserves and economic benefits for the seafood export industry in Vietnam. According to statistics from the Vietnam Association of Seafood Exporters and Producers (VASEP), in the first nine months of 2022, the country's octopus exports accounted for 43.6% of the total export value of squid and octopus, reached 213 million USD [2]. These figures show that octopus is a commodity with high commercial and high export value.

In general, seafood and cephalopods are the main contributors of n-3 polyunsaturated fatty acids (PUFA) in the human diet. Lipids of marine species, such as marine fish or cephalopods, are often characterized by the high content of PUFA. Among polyunsaturated fatty acids, EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3) are the predominant n-3 fatty acids [3, 4]. From the nutritional point of view, less attention has been paid to the fatty acid profile of cephalopods. Therefore, it is vital to determine these species' lipid and fatty acid profiles.

A. neglectus and *C. indicus*, two cephalopod species collected in the Arabian Sea, were found to contain more significant quantities of sulfur-containing amino acids and lysine than other cephalopods, which indicated that the protein could effectively complement the limiting amino acids in our daily diets. The C20–C22 long chain n-3 fatty acids, EPA, and DHA, vital for human health, were found to be predominant in the edible parts of these cephalopod species [5]. Besides nutritional values, *A. neglectus* and *C. indicus* also have pharmacological values. The compounds macrocyclic lactones, which were isolated from *A. neglectus*, exhibited potential radical-scavenging capacities (IC_{50} 0.95–1.73 mM) along with anti-hypertensive activities (IC_{50} 1.12–2.34 mM) against angiotensin-converting enzyme (ACE) [6]. Meanwhile, non-sulfated steroid glycoside was isolated from *C. indicus* (cistoindoside B). It displayed superior anti-inflammatory properties, as acknowledged by its promising 5-lipoxygenase attenuation potential (IC_{50} 2.11 μM), then the 5-lipoxygenase inhibitor drug zileuton (IC_{50} 3.76 μM). The anti-inflammatory properties were corroborated by the excellent antioxidant activities (IC_{50} ~ 1.0–1.5 mM) [7].

Despite reports on the nutritional value as well as biological activities of *A. neglectus* and *C. indicus* in Vietnam, there is currently a need to study the nutritional composition of these two octopus species. Meanwhile, *Amphioctopus neglectus* (Nateewathana & Norman, 1999) and *Cistopus indicus* (Rapp, 1835) are also known as two species of octopus with high economic value, widely distributed in the waters of Vietnam [8]. In this study, we clarified the nutritional value, fatty acid composition, and essential lipid composition of these two species of octopus collected from the waters of Ha Long bay, Quang Ninh province. These scientific proofs explain the high commercial value they bring to the Vietnamese market today.

MATERIALS AND METHODS

Animal materials

Specimens of octopus *Amphiotopus neglectus* (Nateewathana & Norman, 1999) and *Cistopus indicus* (Rapp, 1835) were collected by trawl net in the western coastal area of Ha Long Bay, Quang Ninh province (coordinates: 20°55'49.90"N; 106°53'3.36"E) in May 2022. The obtained octopus samples were stored at -20°C., The scientific names of the samples were identified by Dr. Nguyen Duc The at the Institute of Marine Environment and Resources, Hai Phong.

Methods

Total lipid extraction method

From the fresh octopus samples obtained, total lipids were extracted according to the method of Folch J. F., using the solvent system $CHCl_3$:MeOH with a ratio of 2:1 by volume

[9]. The lipid mass and the fresh sample weight calculated the total lipid content.

Method to determine the composition and content of fatty acids

The lipids were converted into fatty acid methyl esters (FAME) using Carreau and Dubacq's method [10]. The total lipid was methylated to the methyl ester form by the agent 2% H_2SO_4 in MeOH; the temperature for metabolism was 80^{o}C for two hours. Finish the reaction, treat the mixture with distilled water and hexane, shake well, extract the hexane layer, and rotate in a vacuum under reduced pressure to remove the solvent to obtain a methylated product mixture. The product mixture was then purified by thin-layer chromatography (5 µm silica gel, Merck Co., Ltd., USA, 6 × 6 cm) with *n*-Hexane:Et2O (95/5, v/v) solvent system. For confirmation of FA structures, gas chromatography-mass spectrometry (GC-MS) analysis of FAME was carried out with a Hewlett - Packard GC-MS system HP-6890 GC/MSD 5973 (USA) with a dedicated capillary column CP-Sil 88 (100 m/0.25 mm/0.25 µm) and standard system C16:0, C18:0. Helium was used as a carrier gas at a linear flow rate of 60 mL/min. The column temperature gradient was 155–220^{o}C (1.5 ^{o}C/min), speed: 10 ^{o}C/min, 260^{o}C/5 min, split: 1:50, and the injector temperature was 250^{o}C. Identification of fatty acids by specialized software that calculates the conversion through the equivalent retention time value ELC (Equivalent Chain- lengths of methyl ester derivatives of fatty acids). Using standard data spectra from mass spectrometry libraries: WILEY275.L and NIST 98.L.

Method to determine the composition and content of lipid classes

The composition and content of lipid classes were analyzed and determined on pre-coated plates. Total lipids were dotted onto silica gel (6 × 6 cm, Sorbfil, Krasnodar, Russia) in 3 streaks with different concentrations and then deployed in the solvent system n-Hexan: Et_2O:CH_3COOH (70/30/2, v/v/v), visualized with 10% H_2SO_4 reagent in MeOH, dried at 210^{o}C for 20 minutes. The TLC plate was scanned on an Epson Perfection 2400 PHOTO machine (Nagano, Japan) with standard resolution and size. The percentages of the lipid classes in total lipid were determined based on area and color intensity measurements in the image analysis program Sorbfil TLC Videodensitometer DV, Krasnodar, Russia [11].

RESULTS AND DISCUSSION

Total lipid content, composition and fatty acids content

The percentage of total lipid content in males and females of two octopus species was determined based on the total lipid mass obtained and the fresh sample mass. The results are presented in Table 1. The total lipid content of *A. neglectus* (average content of 3.73%) was higher more than 2 times compared to *C. indicus* (average content of 1.48%). For each species, the total lipid content obtained in males was higher than in females. Notably, the lipid content of *A. neglectus* was higher than those of the squid *Loligo chinensis* Gray in Co To (3.51%) [12] and the squid *Loligo chinensis* Gray in Nha Trang (~1 %), two cephalopod species have large output and high export value [13].

Table 1. Total lipid content of the studied samples

Samples	Fresh sample (g)	Total lipid (g)	% of total lipid
A. neglectus (F)	55.21	1.98	3.58
A. neglectus (M)	42.63	1.65	3.87
C. indicus (F)	57.06	0.84	1.47
C. indicus (M)	49.13	0.74	1.50

The fatty acid compositions of cephalopod species under study are presented in Table 2. Both studied species of octopus demonstrated greater quantities of polyunsaturated fatty acids (PUFA) (58.8–73.6%) followed by saturated fatty acids (SFA) (19.5–24.5%) and monounsaturated fatty acids (MUFA) (7.0–16.7%). The highest content of PUFAs was found in females *A. neglectus*, the highest content of SFAs was found in males *C. indicus*,

and the highest content of MUFAs was found in females *C. indicus.* Palmitic acid (16:0) was the predominant SFA found in female and male *A. neglectus* (12.23% and 14.96%, respectively) and *C. indicus* (13.04 % and 14.92%, respectively), followed by stearic acid (18:0) (3.72% and 4.64% in *A. neglectus*, respectively) and (5.00% and 5.89% in *C. indicus*, respectively).

Table 2. Composition and content of fatty acids of *A. neglectus* and *C. indicus* in total lipids

Fatty acids	% of total fatty acids			
	A. neglectus		*C. indicus*	
	F	M	F	M
14:0	1.262	1.098	0.645	0.865
15:0	1.044	0.419	0.550	1.099
16:0	**12.234**	**14.961**	**13.041**	**14.918**
17:0	1.268	2.671	3.726	1.698
18:0	3.716	4.643	5.003	5.891
16:1n-7	1.011	0.678	0.982	0.744
18:1n-7	1.749	2.212	1.677	1.556
18:1n-9	2.446	3.642	3.056	3.469
18:2n-6	2.361	-	0.375	4.060
20:1n-9	1.649	5.820	10.971	1.965
20:3n-3	1.048	1.169	1.622	1.933
20:4n-3	0.762	0.501	0.987	0.512
20:4n-6 (AA)	**8.993**	**8.237**	**13.427**	**17.257**
20:5n-3 (EPA)	**21.535**	**17.673**	**15.906**	**12.759**
22:6n-3 (DHA)	**34.558**	**32.513**	**20.647**	**25.050**
22:5n-3	4.006	2.626	5.261	2.620
22:5n-6	0.359	0.310	-	0.419
Total saturated fatty acids	18.479	23.374	22.415	23.372
Total unsaturated fatty acids	81.521	75.800	75.460	73.444

Notes: F- female; M- male.

The results of the analysis showed that polyunsaturated fatty acids (PUFAs) with high activity, such as docosahexaenoic acid (DHA, ω-3), eicosapentaenoic acid (EPA, ω-3), and arachidonic acid (AA, ω-6) accounted for the highest concentration with respective percentages in the range (20.65–34.56%), (12.76–21.53%), and (8.23–17.26%). These fatty acids are essential for the human body that cannot be synthesized. They can only enter the body through supplemental food. The total PUFA content was significantly greater in *A. neglectus* than in *C. indicus*. The content of PUFAs contributed about 72.86% and 62.53% of the total FAs in females and males of *A. neglectus*, respectively 57.19% and 64.1% in *C. indicus*. The mean total of n-3 PUFA content was more significant than n-6 PUFA in both species. DHA (22:6n-3) was found to be the predominant n-3 PUFA in these two species, followed by EPA (20:5n-3). The aggregate DHA and EPA content of *A. neglectus* were significantly greater than those in *C. indicus*. The major n-6 PUFAs were found to be 18:2n-6 and 20:4n-6. The unsaturated fatty acid 20:4n-6 (arachidonic acid) in *C. indicus* (13.43% (F) and 17.26% (M)) nearly as twice as in *A. neglectus* (8.99% (F) and 8.24% (M)). Arachidonic acid is a precursor for the biosynthesis of prostaglandins and eicotrienes, important hormones in living organisms. Notably, with *A. neglectus* specie, all three components, DHA, EPA, and AA of the females (34.56%; 21.54%; and 8.99%, respectively) were higher than those of the males (32.51%; 17.67%; and; 8.24%, respectively). Meanwhile, for *C. indicus* specie, the DHA and AA compositions of females (20.65% and 13.43%) were lower than those of males (25.05% and 17.26%). This result is also consistent with Chakraborty's results when

studying a group of five cephalopods in the Arabian Sea, including these two species of octopus [5].

The results indicated that two species of octopus studied have high nutritional value, and *A. neglectus* has a higher nutritional value than *C. indicus*. They contain high levels of essential unsaturated fatty acids necessary for human body development, such as DHA, EPA, and AA. They are foods with high value or potential to produce products that support human health. These scientific results prove their high economic value to the commercial and export markets.

Composition and content of lipid classes

The total lipids of the studied samples were analyzed for the composition and content of the lipid classes according to the method described. The results showed that the composition of lipid classes in total lipids of the studied samples consisted of only four classes of substances: polar lipids (PL), sterols (ST), free fatty acids (FFAs), and triacylglycerol (TG). All studied samples showed the absence of two groups of substances, monoalkyl diaylglycerol (MADG) and hydrocarbon-wax (HW), which are typical classes of substances commonly present in the lipid composition of some marine species such as corals and mollusks (2 shells).

Based on the measurement of area and color intensity in the image analysis program Sorbfil TLC Videodensitometer DV (Krasnodar, Russia), the percentages of substance classes in the total lipids of the studied samples were determined and presented in Table 3. The analysis results show that the studied samples have similar content ratios between the classes of substances. In which the class of polar lipids has the highest content (accounting for 44.88–54.14%), followed by sterols (accounting for 31.12–35.95%), free fatty acids (accounting for 5.12–10.58%), and the lowest content of triacylglycerols (accounting for 4.90–9.61%). The relationship between measurements of area, color intensity, and scanned TLC images is shown in Table 4.

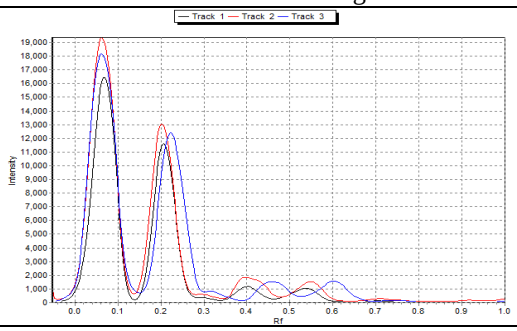
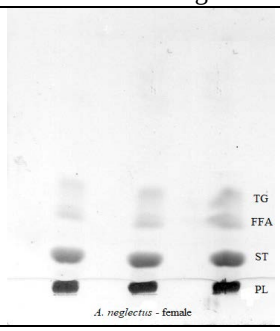
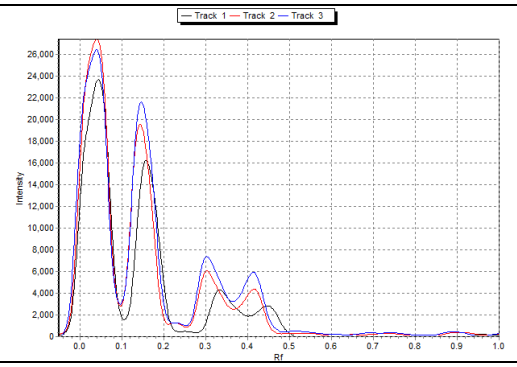
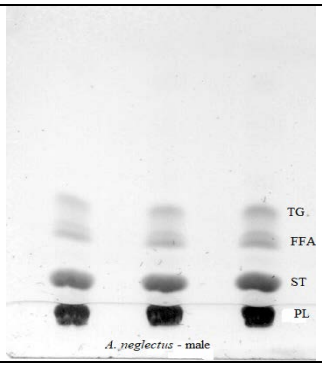
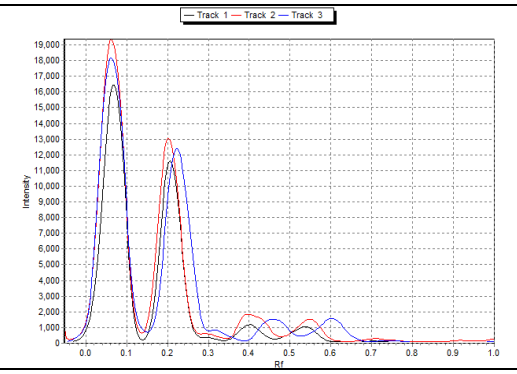
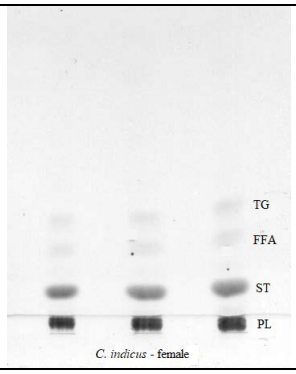
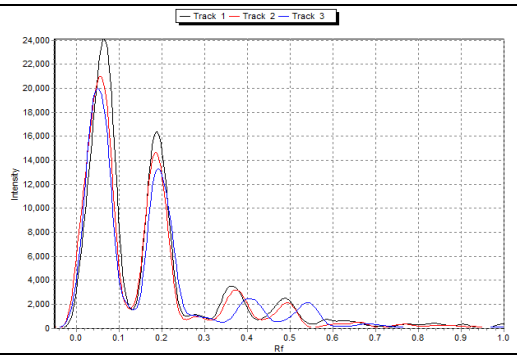
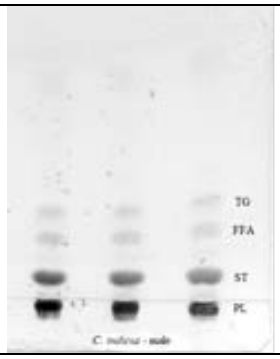
Table 3. Composition and content of lipid classes in studied samples

Name sample	Content of lipid classes (% of total lipid)			
	PL	ST	FFA	TG
A. neglectus (F)	44.88	35.95	9.56	9.61
A. neglectus (M)	50.29	31.12	10.58	8.02
C. indicus (F)	54.14	35.84	5.12	4.90
C. indicus (M)	52.75	34.34	7.53	5.38

Notes: PL- polar lipid; ST- sterol; FFA- free fatty acid; TG- triacylglycerol.

Similar to the composition and content of PUFAs in the studied samples, which all accounted for high content, the content of the PL class also accounted for a high percentage. Obtained results indicated that the research samples have potential for biological applications because the PL class contains mainly components of phospholipids (such as phosphatidylethanolamine (PE), phosphatidyl-choline (PC), phosphatidylserine (PS), phosphatidylinositol (PI), or phosphonolipid ceramide aminoethylphosphonate (CAEP)), a group of substances with a broad biological spectrum and many applications in medicine and pharmacology. In order to determine the composition and content of the groups of substances in the phospholipids class, more in-depth and detailed studies are needed. However, the fact that the studied samples all have a high content of polar lipids shows their biological potential. Although *C. indicus* has a lower proportion of unsaturated fatty acids such as DHA and EPA than *A. neglectus*, the content of their polar lipids (52.75% (male) and 54.14% (female)) is higher than those of *A Amphiotopus neglectus* (50.29% (male) and 44.88% (female)). There was not much difference between the studied samples for the class of sterols. The substance classes FFA and TG of males and females of *Amphiotopus neglectus* were almost twice the times higher than those of *C. indicus*.

Table 4. Relationship of measurement of area, color intensity and scanned TLC images

TLC on VD Chromatogram	TLC Chromatogram
	
Amphioctopus neglectus (F)	
	
Amphioctopus neglectus (M)	
	
Cistopus indicus (F)	
	
Cistopus indicus (M)	

CONCLUSION

Research results show that cephalopods, as a storehouse of essential nutritional elements, are necessary for human nutrition and metabolism. More remarkably, levels of long-chain polyunsaturated fatty acids C20-22 n-3 and n-6, for instance, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid, have demonstrated that these cephalopods are good sources of well-balanced diets. The total lipid content of *A. neglectus* was twice that of *C. indicus*. For each species, the total lipid content obtained in males was higher than in females. All samples' total unsaturated fatty acid content was 3 to 4.5 times higher than the total saturated fatty acid content. Palmitic acid (C16:0) was determined to account for the highest percentage of total saturated fatty acids.

Meanwhile, DHA (22:6n-3) was confirmed to account for the highest percentage of total unsaturated fatty acids, followed by EPA (20:5n-3) and AA (20:4n-6). DHA and EPA contents of *A. neglectus* were significantly higher than those of *C. indicus*. In contrast, the AA content of *C. indicus* was superior to that of *A. neglectus*. The composition of lipid classes in the total lipids of the cephalopods showed only four lipid classes, including polar lipids (PL), sterols (ST), free fatty acids (FFAs), and triacylglycerol (TG), which are both absent from monoalkyl diacylglycerol (MADG) and hydrocarbon-wax (HW) classes, typical lipid classes in marine organisms. In particular, the class of polar lipids with a broad spectrum of activity and many biological effects accounted for the largest concentration in the studied samples (accounting for 45–54%). These results prove that both *A. neglectus* and *C. indicus* have high nutritional and commercial value, providing helpful information for the seafood industry and the utilizing the cephalopod species as potential health foods.

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