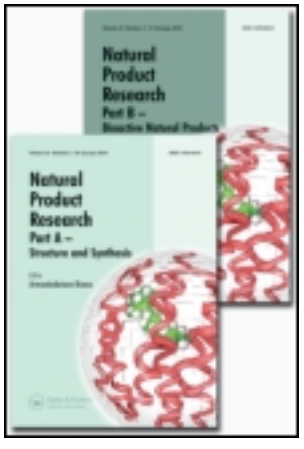

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Fatty acid composition of the edible sea cucumber *Athyonidium chilensis*

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The edible sea cucumber *Athyonidium chilensis* is a fishery resource of high commercial value in Chile, but no information on its lipid and fatty acid composition has been previously reported. Phospholipids were the major lipid contents of the ethanolic extracts of tubules, internal organs and body wall of *A. chilensis*. Saturated fatty acids predominated in tubule phospholipids (40.69%), while in internal organs and body wall phospholipids, the monounsaturated fatty acids were in higher amounts (41.99% and 37.94%, respectively). The main polyunsaturated fatty acids in phospholipids were C20 : 2ω-6, arachidonic (C20 : 4ω-6) and eicosapentaenoic (C20 : 5ω-3) acids. These results demonstrate for the first time that *A. chilensis* is a valuable food for human consumption in terms of fatty acids.

Keywords: sea cucumber; *Athyonidium chilensis*; phospholipids; fatty acids; CG-MS; PUFAs

1. Introduction

Sea cucumbers are one of the most widely distributed species of invertebrates, which are well known to contain biologically active triterpene glycosides (Kalinin, Aminin, Avilov, Silchenko, & Stonik, 2008; Maier, 2008) responsible for the toxicity of some species. On the other hand, many sea cucumber species are edible and of high nutritional value (Kasai, 2003; Mamelona, Saint-Louis, & Pelletier, 2010). These marine organisms are traditionally eaten raw or processed into foods in the Indo-Pacific region, including the Philippines, Malaysia, Japan, Korea and China. Recently, antioxidant activities of fresh and processed extracts of *Cucumaria frondosa* (Mamelona et al., 2007; Zhong, Khan, & Shahidi, 2007) have been reported, suggesting the use of this sea cucumber as a potential source of antioxidants for humans.

As part of our investigations on polar metabolites from cold water echinoderms (Careaga, Bueno, Muniain, Alché, & Maier, 2009; Careaga, Muniain, & Maier, 2011), we studied the content of lipids of the edible sea cucumber *Athyonidium chilensis* (Semper, 1868) (Dendrochirotida, Cucumariidae). This is one of the most abundant species in coastal waters of Chile and a fishery resource of high commercial value (Ruiz, Ibáñez, & Cáceres, 2007). Nevertheless, no studies concerning the lipids and fatty acid composition

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of this sea cucumber have been reported. Nowadays, food composition data are essential for commerce exchange and consumer protection (Bell et al., 2011). Recently, a report has been published on the importance of updating food composition databases in Argentina, Chile and Paraguay (Sammán, Masson, de Pablo, & Ovelar, 2011). The aim of this study was to analyse and compare the lipid contents and fatty acid compositions in the body wall, tubules and internal organs of *A. chilensis* in order to determine its nutritional quality in terms of fatty acids.

2. Results and discussion

Polar lipids (PL) were the major lipid contents of the ethanolic extracts of tubules, internal organs and body wall of *A. chilensis*. The PL contents of the internal organs and tubules were 6.4 and 5.6 times, respectively, that of non-polar lipids (NPL). In the body wall, the PL content was 1.4 times that of the NPL.

Analysis by TLC of NPL fractions revealed the presence of triacylglycerides as the main components. Purification of PL fractions by column chromatography on silica gel and Sephadex LH 60 led to the isolation of phospholipids as the major PL and a minor complex mixture of triterpene glycosides, whose chemical structures were not elucidated due to the low amounts of each glycoside in the mixture. The main phospholipid in internal organs, tubules and body wall was identified as phosphatidylcholine by two-dimensional TLC. Phosphatidylserine and lisophosphatidylcholine were identified as minor components of the phospholipid mixtures. PL, NPL and phospholipids were transmethylated with 4% HCl in CH_3OH to obtain the mixtures of fatty acid methyl esters (FAME), which were analysed by CG-MS. The position of the double bond in the monounsaturated FAME was determined by GC–MS analysis of their DMDS derivatives (Díaz de Vivar, Seldes, & Maier, 2002).

2.1. *NPL fatty acid compositions*

Table 1 shows tubules, internal organs and body wall fatty acid compositions of NPL of *A. chilensis*. A total of 18 fatty acids were identified from tubules, 29 from the internal organs and 36 from the body wall. The fatty acid profiles were dominated by saturated fatty acids (SFAs) in tubules (50.80%) and internal organs (50.39%), while in the body wall, the amounts of SFAs (36.61%) were lower than those of monounsaturated fatty acids (MUFAs) (46.90%). Among the SFAs, palmitic acid (C16 : 0) was the dominant fatty acid in tubules, accounting for 22.40% of the total fatty acids, followed by myristic (C14 : 0) (15.50%) and stearic (C18 : 0) (7.49%) acids. Stearic acid was the major fatty acid in the internal organs (14.25%) and body wall (10.69%). Palmitic (11.23%) and myristic (8.72%) acids were also abundant in the internal organs.

The main MUFAs in tubules were palmitoleic (C16 : 1ω-7) (11.32%), oleic (C18 : 1ω-9) (10.30%) and C-20 : 1ω-9 (7.55%) acids. Palmitoleic acid was present at lower levels in the internal organs (4.34%) and the body wall (2.05%), while vaccenic acid (C18 : 1ω-7) (8.11%) was the dominant MUFA in the internal organs. MUFAs in the body wall accounted for 46.90% of the total fatty acids. The main MUFAs were nervonic acid (C24 : 1ω-9) (7.72%), C23 : 1ω-9 (7.19%), C18 : 1ω-7 (6.26%) and C-20 : 1ω-9 (5.97%) acids. It is noticeable that the high content of C23 : 1ω-9 in NPL of the body wall. Some types of sea cucumber are known to contain considerable quantities of this unusual fatty acid in marine lipids (Kasai, 2003). It has been suggested that C23 : 1ω-9 is synthesised *de novo* from C24 : 1ω-9 by α-oxidation, but not by desaturation of odd-chain SFAs (Drazen, Phleger, Guest, & Nichols, 2008; Kaneniwa, Yutaka, Endo, & Takagi, 1986). In our study, the high level of C24 : 1ω-9 in the body wall (7.72%) would be in accordance

Table 1. Fatty acid composition of tubules, internal organs and body wall of NPL (percentage of total fatty acid).

NPL			
Fatty acid	Tubules	Internal organs	Body wall
14:0	15.50 ± 0.80	8.72 ± 0.50	1.44 ± 0.10
15:0	3.33 ± 0.30	1.98 ± 0.60	0.49 ± 0.07
15:0 br	3.77 ± 0.20	9.45 ± 0.20	3.63 ± 0.30
16:0	22.40 ± 0.10	11.23 ± 0.07	1.97 ± 0.06
16:0 br	1.43 ± 0.10	3.48 ± 0.02	0.68 ± 0.10
16:1(ω-5)	2.08 ± 0.40	0.16 ± 0.40	–
16:1(ω-7)	11.32 ± 0.40	4.34 ± 0.40	2.05 ± 0.08
17:0	2.08 ± 0.10	4.34 ± 0.10	1.07 ± 0.10
17:0 br	2.26 ± 0.01	3.04 ± 0.50	2.61 ± 0.10
17:2	–	–	1.09 ± 0.10
18:0	7.49 ± 0.20	14.25 ± 0.50	10.69 ± 0.07
18:0 br	–	1.38 ± 0.30	0.98 ± 0.10
18:1(ω-5)	0.34 ± 0.20	0.20 ± 0.08	–
18:1(ω-7)	0.69 ± 0.20	8.11 ± 0.08	6.26 ± 0.08
18:1(ω-9)	10.30 ± 0.20	–	2.37 ± 0.08
18:2(ω-6)	–	0.98 ± 0.10	0.77 ± 0.04
19:0	–	2.39 ± 0.30	1.63 ± 0.06
19:0 br	–	1.65 ± 0.20	2.53 ± 0.25
19:1(ω-7)	–	1.35 ± 0.10	0.65 ± 0.12
20:0	–	2.15 ± 0.40	1.82 ± 0.07
20:1(ω-7)	1.45 ± 0.10	0.45 ± 0.30	1.47 ± 0.09
20:1(ω-9)	7.55 ± 0.10	4.52 ± 0.30	5.97 ± 0.09
20:2(ω-6)	3.93 ± 0.08	–	2.00 ± 0.06
20:4(ω-6)	1.12 ± 0.02	2.73 ± 0.20	0.85 ± 0.09
20:5(ω-3)	2.92 ± 0.10	2.71 ± 0.10	0.68 ± 0.09
21:0	–	1.24 ± 0.20	1.41 ± 0.06
21:1(ω-7)	–	–	0.61 ± 0.05
22:0	–	2.11 ± 0.50	4.23 ± 0.05
22:1(ω-7)	–	0.46 ± 0.10	1.63 ± 0.10
22:1(ω-9)	–	0.68 ± 0.10	1.22 ± 0.10
23:0	–	1.98 ± 0.03	1.90 ± 0.07
23:1(ω-9)	–	1.85 ± 0.20	7.19 ± 0.15
24:0	–	–	2.25 ± 0.04
24:1(ω-9)	–	1.85 ± 0.10	7.72 ± 0.40
25:1(ω-9)	–	–	1.16 ± 0.01
αOH 22:0	–	–	4.51 ± 0.40
αOH 23:0	–	–	3.20 ± 0.40
αOH 24:1	–	–	8.60 ± 0.30
br	7.46	19.00	10.43
SFAs	50.80	50.39	36.61
MUFAs	33.73	23.97	46.90
PUFAs	7.97	6.42	5.39

Note: br, branched fatty acids.

with this proposal. Elevated levels of C23 : 1ω-9 and other MUFAs might be related to the maintenance of membrane fluidity at low temperatures and high pressures (Ginger, Santos, & Wolff, 2000).

Polyunsaturated fatty acids (PUFAs) comprised 7.97%, 6.42% and 5.39% of the total fatty acids in tubules, internal organs and body wall, respectively. C20 : 2ω-6 (3.93%) was the main PUFA in tubules, followed by eicosapentaenoic acid (EPA) (C20 : 5ω-3) (2.92%)

and arachidonic acid (AA) (C20 : 4ω-6) (1.12%). The internal organs contained AA (2.73%), EPA (2.71%) and linoleic acid (C18 : 2ω-6) (0.98%), while the body wall contained C20 : 2ω-6 (2.00%) as the main PUFAs, followed by C17 : 2 acid (1.09%) and minor amounts of AA (0.85%) and EPA (0.68%).

In the body wall, it was interesting that the identification of three 2-hydroxy fatty acids: 2-hydroxydocosanoic (αOH 22 : 0) (4.51%), 2-hydroxytricosanoic (αOH 23 : 0) (3.20%) and 2-hydroxytetracosenoic (αOH 24 : 1) (8.60%), which accounted for 16.31% of the total fatty acids. These 2-hydroxy fatty acids have been previously reported in low amounts (0.5–1.2%) in the phospholipid fatty acids of the holothurian *Holothuria mexicana* (Carballeira, Cruz, & Sostre, 1996), while high amounts of αOH 24 : 1 (10.5–14.1%) have been detected in four holothurians of the abyssal eastern North Pacific (Drazen et al., 2008).

Branched chain fatty acids accounted for 7.46%, 19.0% and 10.43% in tubules, internal organs and body wall, respectively, together with odd carbon SFAs (C15 : 0, C17 : 0 and C19 : 0), characteristic of bacterial origin. As detritus is part of the diet of *A. chilensis* (Ruiz et al., 2007), the relatively large amount of normal and branched chain odd SFAs, particularly in the internal organs, is believed to have been derived from this diet.

2.2. *PL and phospholipid fatty acid compositions*

Table 2 shows tubules, internal organs and body wall fatty acid compositions of PL and phospholipids of *A. chilensis*. The fatty acid profiles of PL are reflective of phospholipid fatty acids, since these were the predominant PL. As for NPL, SFAs predominated in tubule phospholipids (40.69%), while in internal organs and body wall phospholipids the MUFAs were in higher amounts (41.99% and 37.94%, respectively) than the SFAs (38.42% and 24.22%, respectively). Stearic acid was the dominant SFA in tubules (15.56%), internal organs (14.18%) and body wall (15.43%) phospholipids, followed by palmitic acid (8.67%) in tubules. Similar results have been reported for phospholipids in *H. mexicana* where stearic and palmitic acids were the most abundant SFAs (Carballeira et al., 1996).

MUFAs in tubule phospholipids were dominated by oleic (10.22%) and C20 : 1ω-9 (12.16%) acids, followed by minor amounts of C18 : 1ω-11 (2.17%) and palmitoleic (1.21%) acids. C18 : 1ω-11 (10.78%) was the dominant unsaturated fatty acid in the internal organs, followed by oleic acid (7.42%), C20 : 1ω-9 (4.94%), C23 : 1ω-9 (3.91%) and palmitoleic (2.52%) acids. The body wall contained a high level of oleic acid (15.38%) together with C18 : 1ω-11 (6.13%), palmitoleic (4.33%), C20 : 1ω-9 (2.66%) and C23 : 1ω-9 (2.54%) acids.

PL and phospholipids in tubules and body wall were richer in PUFAs (23.17–21.16% and 15.53–19.18%, respectively) compared to NPL (7.97% and 5.39%, respectively). The main PUFAs in phospholipids of tubules were C20 : 2ω-6 (6.72%) acid and AA (5.79%), followed by EPA (2.01%) and C22 : 2ω-6 (1.01%). AA (8.70%) and EPA (5.92%) were the dominant PUFAs in body wall phospholipids, followed by C18 : 2ω-6 (2.49%), C20 : 3ω-6 (1.07%) and C20 : 2ω-6 (1.00%) acids. The internal organs phospholipids contained AA (1.84%), C18 : 2ω-6 (1.79%) and EPA (1.55%). AA and EPA have beneficial effects on metabolism and in the prevention of cardiovascular disease (Simopoulos, 1999). High levels of EPA have been associated with tissue repair ability of sea cucumbers (Fredalina et al., 1999).

Four 2-hydroxy fatty acids (αOH 15 : 0, αOH 16 : 0, αOH 18 : 0 and αOH 22 : 0) were detected in low levels (0.1–0.37%) in the PL of tubules, while αOH 15 : 0, αOH 16 : 0 and αOH 22 : 0 were identified in slightly higher levels (0.65–0.98%) in tubule phospholipids.

Table 2. Fatty acid composition of tubules, internal organs and body wall of PL and phospholipids (percentage of total fatty acid).

	PL			Phospholipids		
Fatty acids	Tubules	Internal organs	Body wall	Tubules	Internal organs	Body wall
14 : 0	2.55 ± 0.03	5.35 ± 0.30	1.94 ± 0.07	1.02 ± 0.04	1.88 ± 0.10	0.98 ± 0.01
15 : 0	1.47 ± 0.02	1.26 ± 0.03	2.09 ± 0.20	1.14 ± 0.01	3.32 ± 0.08	1.04 ± 0.04
15 : 0 br	2.53 ± 0.02	8.73 ± 0.01	3.97 ± 0.20	1.02 ± 0.10	1.03 ± 0.10	2.47 ± 0.01
16 : 0	11.01 ± 0.03	11.08 ± 0.10	2.91 ± 0.01	8.67 ± 0.20	3.06 ± 0.10	0.38 ± 0.01
16 : 0 br	0.86 ± 0.05	1.59 ± 0.01	1.03 ± 0.30	1.03 ± 0.30	1.30 ± 0.10	0.73 ± 0.03
16 : 1(ω-5)	–	0.27 ± 0.04	–	–	–	–
16 : 1(ω-7)	2.93 ± 0.03	3.56 ± 0.04	2.54 ± 0.01	1.21 ± 0.01	2.52 ± 0.10	4.33 ± 0.10
17 : 0	0.66 ± 0.01	3.42 ± 0.03	1.83 ± 0.10	3.03 ± 0.10	2.25 ± 0.10	2.20 ± 0.10
17 : 0 br	0.85 ± 0.01	1.34 ± 0.10	2.60 ± 0.10	2.07 ± 0.03	2.31 ± 0.20	4.29 ± 0.08
17 : 1(ω-9)	0.30 ± 0.01	0.54 ± 0.01	–	–	–	–
18 : 0	11.53 ± 0.20	14.17 ± 0.30	13.69 ± 0.10	15.56 ± 0.12	14.18 ± 0.30	15.43 ± 0.10
18 : 0 br	2.02 ± 0.04	0.80 ± 0.20	1.42 ± 0.10	2.21 ± 0.08	2.03 ± 0.20	2.78 ± 0.03
18 : 1(ω-7)	–	0.37 ± 0.03	–	–	–	–
18 : 1(ω-9)	8.38 ± 0.10	4.77 ± 0.10	8.08 ± 0.05	10.22 ± 0.10	7.42 ± 0.30	15.38 ± 0.10
18 : 1(ω-11)	2.61 ± 0.10	3.01 ± 0.09	4.81 ± 0.05	2.17 ± 0.10	10.78 ± 0.30	6.13 ± 0.10
18 : 2(ω-6)	0.69 ± 0.04	1.06 ± 0.10	1.80 ± 0.04	–	1.79 ± 0.10	2.49 ± 0.10
19 : 0	0.70 ± 0.20	3.06 ± 0.01	1.97 ± 0.06	1.61 ± 0.08	4.74 ± 0.30	1.03 ± 0.03
19 : 0 br	–	1.04 ± 0.05	1.29 ± 0.05	–	3.66 ± 0.20	0.88 ± 0.01
19 : 1(ω-9)	0.98 ± 0.01	1.14 ± 0.01	1.26 ± 0.02	1.51 ± 0.06	1.91 ± 0.10	–
20 : 0	1.61 ± 0.04	1.53 ± 0.06	2.44 ± 0.30	1.00 ± 0.02	2.58 ± 0.20	1.36 ± 0.01
20 : 1(ω-7)	1.59 ± 0.20	1.22 ± 0.09	0.77 ± 0.30	1.67 ± 0.05	1.86 ± 0.13	1.66 ± 0.01
20 : 1(ω-9)	13.49 ± 0.20	7.59 ± 0.09	3.88 ± 0.30	12.16 ± 0.05	4.94 ± 0.13	2.66 ± 0.01
20 : 2(ω-6)	6.4 ± 0.08	–	1.66 ± 0.20	6.72 ± 0.10	–	1.00 ± 0.01
20 : 3(ω-6)	0.49 ± 0.08	2.56 ± 0.20	1.36 ± 0.03	–	–	1.07 ± 0.03
20 : 4(ω-6)	6.27 ± 0.10	2.02 ± 0.08	10.24 ± 0.04	5.79 ± 0.10	1.84 ± 0.10	8.70 ± 0.02
20 : 5(ω-3)	5.93 ± 0.30	1.85 ± 0.15	6.1 ± 0.03	2.01 ± 0.03	1.55 ± 0.05	5.92 ± 0.05
21 : 0	0.74 ± 0.10	0.82 ± 0.10	0.90 ± 0.08	1.0 ± 0.20	1.37 ± 0.20	1.19 ± 0.10
21 : 1(ω-14)	0.44 ± 0.20	0.76 ± 0.10	0.55 ± 0.08	0.82 ± 0.07	0.91 ± 0.10	1.02 ± 0.10
22 : 0	0.38 ± 0.06	2.34 ± 0.08	2.16 ± 0.02	1.07 ± 0.10	2.02 ± 0.02	0.61 ± 0.02
22 : 1(ω-7)	0.74 ± 0.03	2.62 ± 0.04	0.60 ± 0.02	–	2.69 ± 0.06	0.73 ± 0.05
22 : 1(ω-9)	1.08 ± 0.03	0.12 ± 0.04	0.33 ± 0.02	0.96 ± 0.01	2.15 ± 0.06	2.30 ± 0.05
22 : 2(ω-6)	1.84 ± 0.04	0.69 ± 0.10	–	1.01 ± 0.06	–	–
22 : 6(ω-3)	1.55 ± 0.20	0.65 ± 0.02	–	–	0.75 ± 0.10	–
23 : 0	0.71 ± 0.10	0.39 ± 0.10	1.3 ± 0.03	1.02 ± 0.07	3.02 ± 0.36	–
23 : 1(ω-7)	–	0.27 ± 0.20	–	–	–	–
23 : 1(ω-9)	0.83 ± 0.01	2.26 ± 0.20	2.08 ± 0.05	0.56 ± 0.05	3.91 ± 0.10	2.54 ± 0.04
24 : 0	0.38 ± 0.08	0.57 ± 0.10	0.47 ± 0.06	1.48 ± 0.06	–	–
24 : 1(ω-9)	1.37 ± 0.02	3.68 ± 0.10	2.44 ± 0.10	1.01 ± 0.02	2.9 ± 0.09	1.19 ± 0.07
25 : 0	–	–	–	0.71 ± 0.04	–	–
26 : 0	–	–	–	0.97 ± 0.01	–	–
αOH 15 : 0	0.27 ± 0.01	–	–	0.98 ± 0.05	–	–
αOH 16 : 0	0.32 ± 0.10	–	–	0.78 ± 0.01	–	–
αOH 18 : 0	0.37 ± 0.20	–	–	–	–	–
αOH 22 : 0	0.1 ± 0.10	–	–	0.65 ± 0.02	–	–
br	6.26	13.50	10.31	6.33	10.33	11.15
SFAs	32.80	43.99	31.70	40.69	38.42	24.22
MUFAs	34.74	32.18	27.34	32.29	41.99	37.94
PUFAs	23.17	8.83	21.16	15.53	5.93	19.18
Heptadecanal	0.70 ± 0.08	0.81 ± 0.10	0.75 ± 0.10	1.07 ± 0.06	0.90 ± 0.12	0.61 ± 0.01
Octadecanal	2.02 ± 0.04	0.71 ± 0.10	6.51 ± 0.10	3.00 ± 0.05	2.10 ± 0.12	5.09 ± 0.01
Nonadecanal			2.01 ± 0.03	0.94 ± 0.01		1.61 ± 0.10

Note: br, branched fatty acids.

These 2-hydroxy fatty acids are characteristic of echinoderm sphingolipids and have previously been identified in the glucosylceramides of the starfishes *Allostichaster inaequalis* (Díaz de Vivar et al., 2002) and *Cosmasterias lurida* (Maier, Kuriss, & Seldes, 1998) and in the galacto- and glucosylceramides of the holothurians *Bohadschia argus* (Ikeda et al., 2009) and *Stichopus japonicus* (Kisa, Yamada, Kaneko, Inagaki, & Higuchi, 2005). Heptadecanal (17 : 0) was identified in low amounts in tubules (0.70–1.07%), internal organs (0.81–0.90%) and body wall (0.61–0.75%) PL and phospholipids. Octadecanal (18 : 0) was abundant in PL (6.51%) and phospholipids (5.09%) of body wall. Nonadecanal (19 : 0) was identified in tubule (0.94%) phospholipids and body wall PL (2.01%) and phospholipids (1.61%). These aldehydes most likely arise from PC plasmalogens (Sugiura, Fukuda, Miyamoto, & Waku, 1992).

Branched chain fatty acid profiles in PL and phospholipids were similar to those in NPL, except for the internal organs where lower levels of branched chain fatty acids (10.31% and 11.15%) were detected in comparison to NPL (19.0%).

According to the results of our study, the fatty acid composition in *A. chilensis* varied depending on the kind of lipids and the tissue selected. Compared with abyssal, tropical and temperate sea cucumbers (Drazen et al., 2008; Svetashev, Levin, Cham, & Do, 1991), *A. chilensis* had higher amounts of SFAs and MUFAs, but lower amounts of PUFAs. These differences are most probably due to different feeding habits. Most animals are unable to synthesise long chain PUFAs such as EPA, AA and DHA, which originate from phytoplankton and some bacteria and are transferred through the food web (Volkman, Jeffrey, Nichols, Rogers, & Garland 1989). Therefore high levels of these fatty acids are suggestive of herbivory. On the contrary, long chain MUFAs such as oleic (C18 : 1ω-9), eicosenoic (C20 : 1) and docosenoic (C22 : 1) acids indicate carnivorous dietary input (Virtue, Mayzaud, Albessard, & Nichols, 2000). Recently, it has been established that *A. chilensis* feeds mainly on macroalgae and invertebrates as well as on microalgae and detritus (Ruiz et al., 2007). Our results on the fatty acid compositions of lipids in tubules, body wall and internal organs of *A. chilensis* are reflective of this diet.

The ω-3/ω-6 ratios in PL and phospholipids of tubules (0.48 and 0.15), internal organs (0.39 and 0.63) and body wall (0.41 and 0.45) in *A. chilensis* are similar to those reported for tropical fresh sea cucumbers (0.41–0.89; Drazen et al., 2008) and dried forms of sea cucumbers used as seafood (0.25–0.61; Wen, Hu, & Fan, 2010). This first study on the composition of lipids and fatty acids of *A. chilensis* revealed that this sea cucumber, and particularly its body wall, has a high nutritional value for human consumption due to its content in PL, mainly phospholipids, with high proportions of oleic acid and the polyunsaturated AA and EPA. Moreover, the ratio of palmitic and stearic acids content (0.03) is very favourable in phospholipids of the body wall of *A. chilensis,* since stearic acid is considered much less hypercholesterolemic than palmitic acid (Grundy, 1994).

3. Experimental

3.1. *Biological materials*

Sea cucumbers of about 8–22 cm long were collected from the sediment intermareal of Las Cruces, Chile (33 29′40″S, 71 37′30″W) and frozen immediately until analyses. The organisms were collected and identified by Dr C. Espoz and Dr C. Muniain. A voucher specimen is preserved at the Museo de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN 37461).

3.2. *Extraction and purification procedures*

Seven specimens of *A. chilensis* (1 kg) were dissected in body wall (muscle, connective tissue and skin), tubules and internal organs (including gonads) and pooled together in glass containers. Each part was homogenised in EtOH and centrifuged. The EtOH extract was evaporated and the aqueous residue was passed through an Amberlite XAD-2 (Sigma) column and eluted with distilled water (until a negative reaction of chloride was observed), followed by MeOH. The MeOH eluates were evaporated under vacuum to give glassy materials which were partitioned between MeOH : H_2O (9 : 1) and cyclohexane to yield (PL) and (NPL). Each NPL fraction was analysed by TLC on precoated Si gel F_{254} using cyclohexane : acetone (70 : 30) as the solvent mixture. Each PL fraction was chromatographed on silica gel column (0.063–0.200 mm) using CH_2Cl_2 : MeOH : H_2O mixtures with increasing amounts of MeOH and H_2O, and finally MeOH as eluents. Fractions eluted with CH_2Cl_2 : MeOH : H_2O (65 : 35 : 4) contained a mixture of phospholipids and minor triterpene glycosides as determined by TLC C_{18} (MeOH : H_2O (70 : 30)). Each mixture was repurified by Sephadex LH 60 using MeOH as eluent. Then, each purified phospholipid fraction was analysed by two-dimensional TLC on precoated Si gel F_{254} using $CHCl_3$: MeOH : 28% NH_3 (65 : 35 : 5) as the first elution mixture and $CHCl_3$: acetone : MeOH : AcOH : H_2O (50 : 20 : 10 : 10 : 5) as the second one. Standards of phospholipids (Sigma) were run at identical conditions (Christie, 2003).

3.3. *Fatty acid analysis*

FAME of NPL, PL and phospholipids were prepared by reaction with 4% HCl in CH_3OH at 70°C for 2 h. After cooling, water was added and the FAME were extracted with $CHCl_3$ and purified through a 500 mg Strata^{TM} SI-1 cartridge of silica gel (55 μm; Phenomenex) with cyclohexane : acetone (90 : 10) as the solvent system. The purified FAME were analysed by gas chromatography coupled to mass spectrometry on a Shimadzu GCMS-QP5050 A (Shimadzu Corporation, Japan) equipped with an Ultra 2 cross-linked methyl silicone fused silica capillary column (50 m × 0.20 mm I.D., 0.11 μm thickness; Agilent Corporation, USA). Helium was the carrier gas. Both injector and detector temperatures were set at 280°C; oven temperature was programmed from 100°C to 280°C at a rate of 5°C min^{-1} and 10 min at 280°C. Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic laboratory standards. Peak area was quantified using methyl tridecanoate (Sigma) as internal standard and expressed as percentage of total fatty acids.

3.4. *Synthesis of DMDS derivatives*

Each sample (5 mg) of FAME of NPL, PL and phospholipids was dissolved in carbon disulfide (0.5 mL) and DMDS (0.5 mL) and iodine (2.5 mg) were added to the solution. The mixture was kept at 60 C for 40 h in a small-volume sealed vial. The reaction was quenched with aqueous $Na_2S_2O_3$ 5%, and the mixture was extracted with cyclohexane (1.0 mL). The extract was evaporated to dryness under reduced pressure and the residue (DMDS derivative) was redissolved in CH_2Cl_2 and analysed by GC-MS on a Shimadzu GCMS-QP5050 A using an Ultra 2 column (50 m × 0.20 mm I.D., 0.11 μm thickness), with helium as the carrier gas. Both injector and detector temperatures were set at 280 C; oven temperature programmed from 100°C to 280°C at a rate of 5°C min^{-1} and 20 min at 280 C.

3.5. *Statistical analysis*

Statistical analyses were performed using Statistix 9 for Windows. All analytical determinations were performed in triplicate and the mean values were reported.

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