Fatty Acid Profile and Total Lipid Content of *Chionoecetes opilio* Shells

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Abstract: In the present study, the Soxhlet method was used to determine the total lipid content of *Chionoecetes opilio* shells. Several parameters were optimized in order to achieve the maximum extraction efficiency.

The fatty acid profile was determined by gas chromatography-flame ionization detection using a one-step-extraction-methylation method to obtain fatty acid methyl esters. GC-MS was used as a confirmatory technique.

Twenty-one fatty acids were identified, and as it was expected, a high content of ω-3 polyunsaturated fatty acids were found, representing 36 % of the total fatty acid content.

The results obtained indicate that snow crab shells might be considered as an innovative source of ω3 long chain polyunsaturated fatty acids for aquaculture feeding purposes.

Keywords: Lipids, Soxhlet, Fatty acids, GC-MS, Chionoecetes opilio.

1. INTRODUCTION

The Snow crab, *Chionoecetes opilio* (Brachyura: Majidae), is an important commercial species in the northwest Atlantic. Crabs, among many others invertebrates, are considered to be important shell fishery products [1], and are widely used as food and feed supplements throughout the world. The demand for products derived from fishing has increased considerably; this has prompted a sustained upward development in aquaculture activity in the last decades. Fatty acids, such as eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and arachidonic acid, are considered essential for marine fish and must be provided in the diet. Marine species are one of the main sources of polyunsaturated fatty acids [2-9].

Several procedures for total lipid extraction (Soxhlet, Bligh & Dyer, Rosse-Gottlieb, etc.) have been described in the literature [10-12]; however there is no unanimous opinion on the most convenient technique. Most of the commonly used techniques are time-consuming, laborious, and large volumes of toxic solvents are required. More recently, supercritical fluid extraction (SFE) has been reported in the bibliography as an excellent alternative to the conventional techniques [13, 14].

The study of total fat content as well as the fatty acid composition, especially polyunsaturated fatty acids (PUFAs) and ω-3 in seafood products is of great interest due to their beneficial effects on coronary diseases [7, 9, 15]. The technique most commonly used for fatty acids analysis is the gas chromatography coupled with a flame ionization detector (GC-FID).

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The major aim of this study was to demonstrate the nutritional value of *C. Opilio* shells for use as feed in fish farms. As a results, the total lipid content and the fatty acid composition of *Chionocetes Opilio* shells were investigated.

2. MATERIALS AND METHODOLOGY

2.1. Samples

Crabs *(Chinoecetes Opilio)* were collected from the North Atlantic coastal region between Greenland and Canada in November 2005. The edible parts were removed manually. The shells were frozen in the ship and transferred to our laboratory. After defrosting, the shells were dried in a vacuum oven at 50ºC until constant weight was reached. Finally, the samples were powdered and stored in the dark.

2.2. Chemicals

Fatty acid methyl ester mix (PUFA No 3); cis-11, 14-eicosadienoic acid methyl ester; cis-8, 11, 14-eicosatrienoic acid methyl ester and cis-11, 14, 17-eicosatrienoic acid methyl ester were obtained from Supelco (Bellefonte, PA, USA). Methanol, petroleum benzine, HCl, CO_3K_2, and SO_4Na_2 were purchased from Merck (Darmstadt, Germany). Toluene was obtained from Sigma-Aldrich (EEUU). All other reagents were of analytical grade. Ultrapure water was obtained from a Milli-Q water purification system Millipore (Bedford, MA, USA).

2.3. Lipid Extraction

Total fat was extracted according the standard AOAC method [16]. One g of dry and homogenized samples, 10 g sea sand, and 200 ml petroleum benzine were extracted by Soxhlet for 7 hours. Then, the solvent was evaporated at 35 ºC using a rotary evaporator. Finally, the lipid content of samples was determined gravimetrically.

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2.4. Fatty Acid Analysis by GC-FID

The samples were processed using an extraction procedure which involves extraction-methylation in a single step [17]. About 0.4 g of each sample was weighed in a 160 x 16 mm Pyrex tube fitted with a PTFE-lined screw cap. Then, 2 mL of toluene and 3 mL of freshly prepared 5 % methanolic HCl were added. After carefully mixing the contents and flushing the headspace of the vial with nitrogen, the tubes were heated for 2 h in a water bath at 70 ºC. K_2CO_3 (6%) solution (5 mL), toluene (1 mL) and 1 mL of nonadenoic acid methyl ester (I.S. toluene solution) were added after cooling the tubes.

The tubes were vortex-mixed and immediately centrifuged at 139.5 g for 5 min in a Hettich EBA 12 centrifuge to separate the phases. The organic layer was dried with anhydrous Na_2SO_4 and 1 µL of liquid phase was injected into the chromatograph.

The fatty acid methyl esters analysis was performed with a Fisons GC-8000 (Manchester, UK) fitted with a split-splitless injector and a Fisons EL-980 flame ionization detector (FID) equipped with a DW-Wax capillary column (60 m X 0.32 mm i.d. fused-silica column coated with a 0.25 µm polyethylene glycol film) (J&W Scientific, Folson, CA, USA). Peak areas were processed using Chrom-Card for Windows (Carlo Erba) software (version 1.18). Samples (1 µL) were injected with a split ratio of 1:15 at a column temperature of 160 ºC and an injector temperature of 250 ºC. The carrier gas used was helium at a flow-rate of 1.35 ml/min, an average linear velocity of 22.76 cm/sg and a head pressure of 15 p.s.i. One minute after injection, the temperature was raised at 3.5 °C/min to 230°C and held for 20 min. The detector temperature was 260 °C. The fatty acid methyl esters profile of all samples were identified by comparison of their retention times with standards.

2.5. Fatty Acid Analysis by GC-MS

For mass spectra analysis, a Fisons MD 800 (Manchester, UK) mass detector and Masslab software (version 1.4) were used. The mass spectrometer operated under the following conditions: ion ionization voltage, 70 eV, mass range, 50-450 *m/z* and scan and inter-scan delay times of 0.45 sg and 0.05 sg, respectively. The oven programme temperature was the same as the methodology described under the GC-FID analysis.

The compounds were identified by comparison of their mass spectra with the Wiley (New York, USA) mass spectrum library (version 1.4).

3. RESULTS AND DISCUSSION

3.1. Total Lipid

Since the selection of the organic phase is a critical step in the Soxhlet extraction different organic solvents were tested; hexane, dichloromethane and petroleum benzine. The best results were achieved when using petroleum benzine. The yield of total lipids obtained in samples tested was around 12 % higher than the yields obtained with the other solvents. In addition, the evaporation of the solvent was shorter because of the lower boiling point of petroleum benzine. Soxhlet extraction is a convenient automated method for lipid extraction from solid samples [10, 18]. Katikou and Robb [18] also used petroleum benzine to extract the fat from salmon samples. In this study, the authors compared the CEM rapid extraction method with the soxhelt for the determination of lipid content in fillets of farmed Atlantic salmon. The lipid values obtained with the soxhelt method were higher than those obtained with the CEM method, although the differences were not significant.

The total lipid yield obtained for 40 samples, expressed as g/100g of dry weight, was (21.9±8.4) (mean±SD). In the Fig. (**1**), the percentage of fat obtained of each sample analysed is presented. As can be seen, the fat content in the samples varied from 2.53 % (sample 6) to 37.86 % (sample 33).

3.2. Fatty Acids

In the second part of the work, the fatty acid methyl esters (FAMEs) profile was determined. Although there are several studies on the fatty acid composition of different species of crabs [19-21], no information about the content of fatty acids of snow crab shell is available.

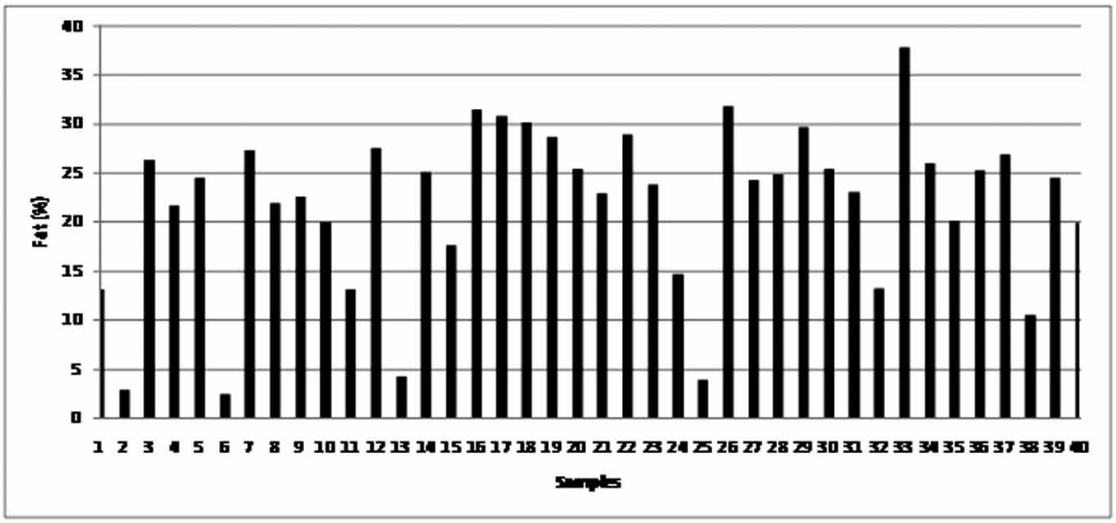

Fig. (1). Total lipid content of 40 samples analysed.

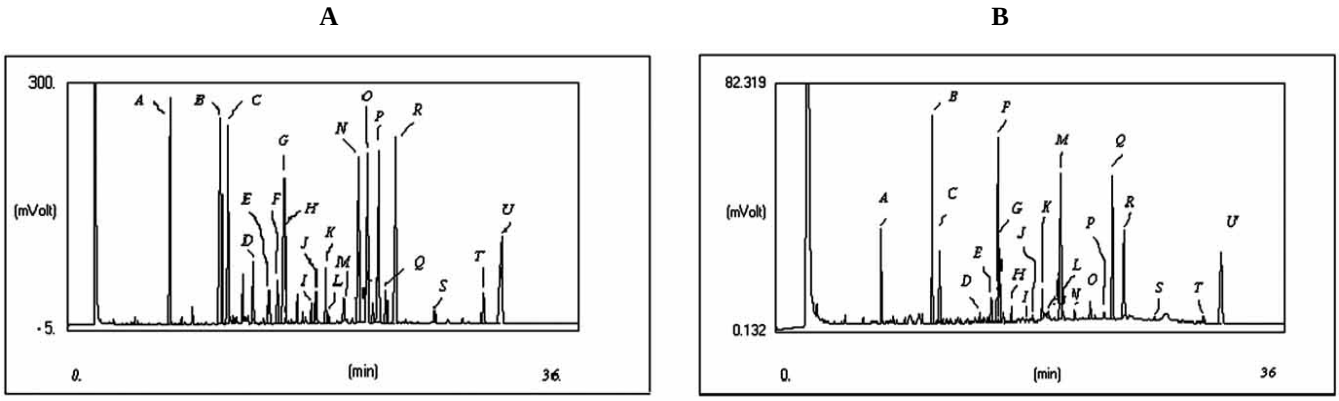

Fig. (2). GC chromatograms of: **A**) standards of fatty acid methyl esters. **B**) Snow crab shells. Peaks: (A) C14:0; (B) C16:0; (C) C16:1ω7; (D) C16:2ω4; (E) C18:0; (F) C18:1ω9; (G) C18:1ω7; (H) C18:2ω6; (I) C18:3ω4; (J) C18:3ω3; (K) C18:4ω3; (L) C20:0; (M) C20:1ω9; (N) C20:2ω6; (O) C20:4ω6; (P) C20:3ω3; (Q) C20:4ω3; (R) C20:5ω3; (S) C22:0; (T) C22:5ω3; (U) C22:6ω3.

An extraction procedure which involves extraction-methylation in a single step was selected because of its reported advantages, rapidity, simplicity, and low cost [22, 23].

Fig. (**2**) shows a chromatogram of a standard solution composed by the fatty acid methyl ester mix (PUFA No 3) and cis-11, 14-eicosadienoic acid methyl ester; cis-8, 11, 14-eicosatrienoic acid methyl ester and cis-11, 14, 17-

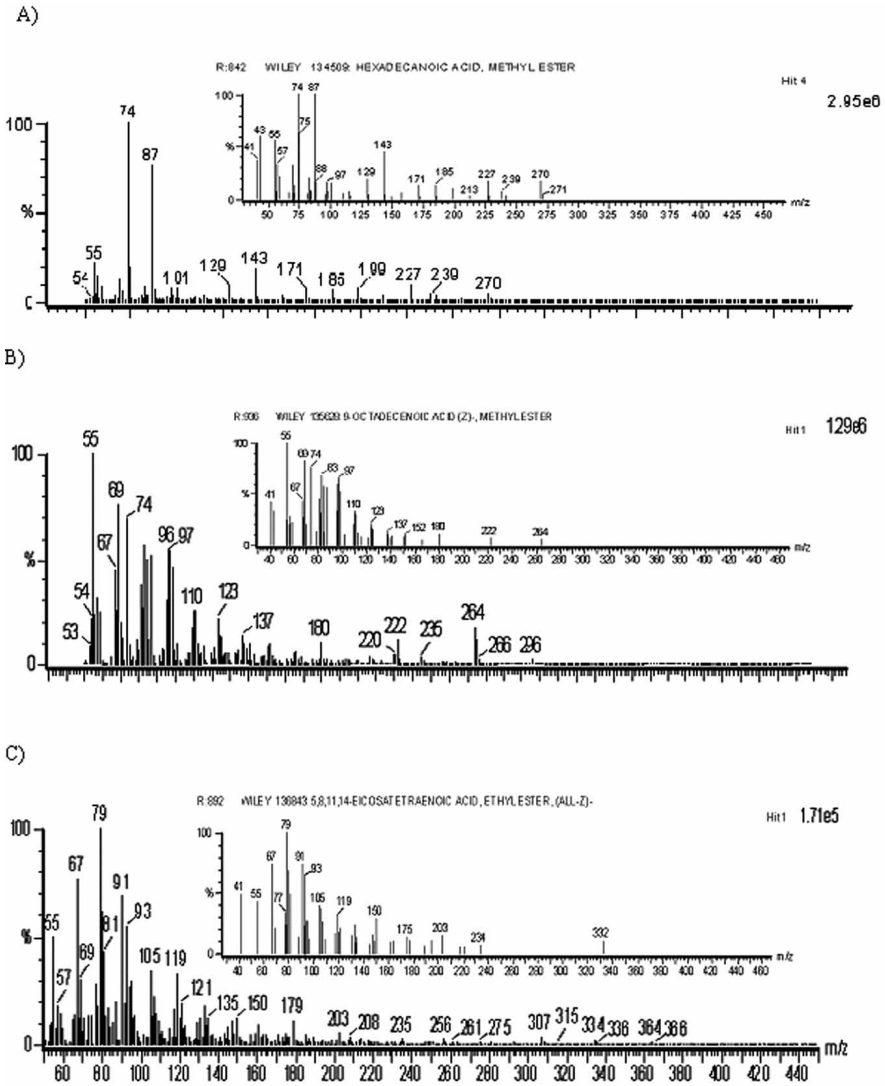

Fig. (3). GC-MS spectra of C16:0 (A), C18:1ω9 (B) and C20:4ω3 (C).

Table 1. Retention Time and Fatty Acid Distribution, Expressed as (g /100g Dry Weight) in Total Lipids, of *Chionoecetes opilio* Shells

Peak	Fatty acid	Retention Time	% (Mean±SD)
A	C14:0	7.01	4.79±1.13
B	C16:0	10.46	13.19±1.11
C	C16:1 ω7	10.95	5.43±1.06
D	C16:2 ω4	13.69	0.74±0.51
E	C18:0	14.44	2.24±0.59
F	C18:1 ω9	14.89	14.71±2.88
G	C18:1 ω7	15.02	4.56±0.93
H	C18:2 ω6	15.81	1.13±0.15
I	C18:3 ω4	17.01	0.06±0.05
J	C18:3 ω3	17.17	0.33±0.16
K	C18:4 ω3	17.83	1.10±0.49
L	C20:0	18.21	0.23±0.21
M	C20:1 ω9	19.09	14.52±3.98
N	C20:2 ω6	20.03	0.64±0.24
O	C20:4 ω6	21.08	1.34±0.76
P	C20:3 ω3	22.02	0.53±0.11
Q	C20:4 ω3	22.60	11.63±2.43
R	C20:5 ω3	23.35	10.42±2.27
S	C22:0	25.44	0.52±0.36
T	C22:5 ω3	28.74	1.21±0.59
U	C22:6 ω3	29.95	10.67±1.69

((n=40) ±S.D).

eicosatrienoic acid methyl ester (**A**) and a chromatogram of the fatty acids methyl esters obtained from a Chionocetes opilio sample (**B**).

Identification of fatty acids were based on the comparison of the retention time with an external standard (PUFA No 3) solution and confirmed in the sample extracts by GC-MS. The GC-MS spectra corresponding to C16, C18:1ω9 and C20:4ω3 are presented in Fig. (**3**).

The fatty acid distribution in total lipids of the *Chionoecetes opilio* shells is shown in Table **1**. Similar amounts of polyunsaturated fatty acids (39.80%) and monounsaturated fatty acids (39.22%) were found. The percentage of saturated fatty acids was the lowest representing 21% of the total fatty acid content (Table **1**).

The main saturated fatty acid is palmitic C16:0 which contributes more than 50 % of the total saturated fatty acids (13.19 %), these results are in accordance with those reported in shrimp by-products [24, 25] and in crab meat [19-21]. The oleic acid C18:1ω9 (14.71 %) and the eicosaenoic acid C20:1ω9 (14.52 %) were also the dominant monounsaturated fatty acids in snow crab shells [19-21, 24, 25].

The fatty acid profile of *Chionoecetes opilio* shells was dominated by polyunsaturated fatty acids, especially ω3 (35.89 %), with a 11.63 % of eicosatetraenoic acid, 10.67 % of docosahexaenoic acid (DHA), and 10.42 % of eicosapentaenoic acid (EPA). Çelik *et al*. [20] analysed the fatty acid profile in claw, breast and hepatopancreas of blue crab, and they found that the ω3/ω6 relationship was higher in the breast (3.18) than in the claw (2.52) or hepatopancreas (1.59).

A ω3/ω6 fatty acids ratio of 0.45 was reported by Chen *et al*. [19] in Chinese mitten crab. In conclusion, the results obtained indicate that snow crab shells might be considered as an innovative source of ω3 long chain polyunsaturated fatty acids for aquaculture feeding purposes.

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