Food Chemistry 111 (2008) 930–933

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fatty acid composition of green crab (*Carcinus mediterraneus*) from the Tunisian mediterranean coasts

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ARTICLE INFO

Article history:
Received 6 October 2007
Received in revised form 29 April 2008
Accepted 1 May 2008

Keywords:
Green crab
Fatty acids
Hepatopancreas
Claw meat
Proximate composition

ABSTRACT

Green crab (*Carcinus mediterraneus*) was analysed for proximate and fatty acid composition. The yields of crab claw meat and hepatopancreas were 24.9–26.1% and 8.8–9.2%, respectively. Crude protein (NX6.25) and crude fat contents of crab claw meat were 17.8–18.2% and 0.85–1%, respectively, on a dry weight basis, while those of hepatopancreas were 13–14% and 21.8–22.7%, respectively. The fatty acid (FA) profiles were significantly different between claw meat, and hepatopancreas of the crab. The percentage of total saturated fatty acids was higher in the hepatopancreas (25.15–26.24% of total FAs) than in the claw meat (22.58–23.49% of total FAs). The main saturated fatty acids were palmitic acid (16:0) and stearic acid (18:0). Palmitic acid represented 11.5–12.45% and 11–11.5% of the total FAs in the hepatopancreas and in the claw meat, respectively. The percentages of stearic acid were 7.8–8.3% and 7–7.3% in the hepatopancreas and in the claw meat, respectively. Meanwhile, oleic acid (18:1) was the dominant monounsaturated fatty acid which represents 16.15–16.85% and 15.4–15.7% of the hepatopancreas and the claw meat total FAs, respectively. The dominant PUFA was arachidonic acid (20:4n-6) in both claw meat and hepatopancreas. The content of arachidonic acid (20:4n-6) was higher in the hepatopancreas (13–13.5%) than in the claw meat (10.5–11.8%).

1. Introduction

Marine organisms and particularly crustaceans have the potential of delivering valuable nutritive products. Crustaceans occupy a huge variety of ecological nich. Commercial fishing is the most common source of crustaceans and other marine organisms. Using the species captured in coastal areas, green crab (*Carcinus mediterraneus*) was examined as one of the Tunisian coastal fisheries. The biochemical compositions of different crab species have been reported to be different in various parts of the world (Chaufan et al., 2002; Krzynowek, Wiggin, & Donahue, 1982; Naczk, Williams, Brennan, Liyanapathirana, & Shahidi, 2004; Siddiquie, Akbar, & Qasim, 1987; Skonberg & Perkins, 2002).

Polyunsaturated fatty acids (PUFAs) have been recognized to have special pharmacological and physiological effects on human health (Haines et al., 1986; Siscovick et al., 1995). In fact, they were benificial for the reduction of coronary artery disease (Skonberg & Perkins, 2002), so they are commercialized as nutraceuticals under the form of capsules or used as ingredient in infant food products.

Many authors have recently investigated the fatty acid profiles of crab tissues in various parts of the world (Celik et al., 2004; Chen, Zhang, & Shresta, 2007). The first step in exploring the economic potential of the green crab and reports on its proximate, amino acid and fatty acid compositions was investigated by Naczk et al. (2004). No informations are available about fatty acid composition of green crab from Tunisian coasts. A determination of the fatty acid profiles of Tunisian green crab tissues such as muscle and hepatopancreas, might give information to consumers, to better know how healthy it is for human consumption. The present study aims to evaluate the fatty acid profiles of green crab caught in Mediterranean Tunisian coasts.

2. Material and methods

2.1. Human pancreatic juice

Human pancreatic juice was a generous gift of Drs. F. Carriere and A. De Caro (Marseille, France). The pancreatic juice was collected from a healthy volunteer at the "La tinome Hospital" (Marseille, France), under the supervision of Dr. René Laugier.

Abbreviations: FAMEs, Fatty acid methyl esters; PUFAs, Polyunsaturated fatty acids; HPL, Human pancreatic lipase; FA, Fatty acid.

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doi:10.1016/j.foodchem.2008.05.007

2.2. Green crabs

Green crabs (*C. mediterraneus*) were collected from four sites of Tunisian mediterranean coasts: "Site1: sidi Mansour, Site2: Kerkenah, Site3: Cheba and Site4: Awabed", using cylindrical traps which were 50 cm in length, 25 cm in width, constructed of a coated 1 × 1 cm wire mesh. Traps were baited with mackerel and deployed at depths of 2 m. Soak time was 4 h, after which the traps were hauted and the crabs were placed on crushed ice until euthanized by freezing. Meat from claw and hepatopancreas were separated manually. In each case, claw meat and hepatopancreas of 50 crabs were blended to comprise a sample then stored at −20 °C until analysed for moisture, lipid, crude protein and fatty acid compositions.

2.3. Proximate composition analyses

Moisture content was determined by drying the sample in an oven at 105 °C until a constant weight was obtained (AOAC, 1990). Crude protein content was determined by the Kjeldahl method (AOAC, 1990), and a conversion factor of 6.25 was used to convert total nitrogen to crude protein. Fat content was determined by using the Soxhlet extraction method (AOAC, 1990). Total lipid was separated in to neutral lipids (steryl esters (SE), triacylglycerol (TG), cholesterol (CHL), diacylglycerol (DG), free fatty acids (FFA) and monoacylglycerol (MG) and polar lipids (phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), phosphatidyl-inositol (PI) and phosphatidyl-serine (PS)) by the solvent method with petroleum ether as basal phase and 95% methanol as recovery phase (Skipski & Braclay, 1969).

2.4. Fatty acids analysis

Fatty acids were extracted and fatty acids methyl esters (FAMEs) were prepared according to the method of Metcalfe et al. (1966). After lipid extraction, using the Soxhlet method and saponification, fatty acids were esterified. FAMEs were finally extracted using methanol.

FAMEs, were analysed by gas chromatography using a Shimadzu gas chromatograph (GC-17A) equipped with polar capillary column (DB-WAX, 30 m length, 0.25 mm I.D., 0.25 microm film thickness; Supelco). The oven temperature was programmed from an initial temperature of 150 °C (0.5 min hold), rising to 200 °C at 6 °C/min, then rising to 230 °C at 4 °C/min, and held isothermal (250 °C) for 15 min. Nitrogen was used as a carrier gas at a flow rate of 1 ml/min. The injection port and the flame ionization detector were maintained at 250 °C. Identification was made by comparison of retention times to those of authentic standards.

2.5. Statistical analyses

All analyses were repeated three times, expect yields ($n = 10$). Results were expressed as mean values ± standard deviation (SD) ($n = 3$). The differences were calculated using one-way analysis of variance (ANOVA), and statistically significant differences were reported at $p < 0.05$. Data analyses were done with the use of SPSS 10.0 software.

2.6. Determination of lipase activity

The lipase activity was measured titrimetrically at pH 8 and 37 °C with a pH-stat, using olive oil emulsion or crab lipid extracts as substrates. Lipolytic activity was expressed as international units. One unit corresponds to 1 μmol of fatty acid released per minute. Specific activities are expressed as units/mg of proteins.

3. Results and discussion

The proximate composition of green crab is shown in Tables 1 and 2. There were significant differences ($p < 0.05$) in yields, moisture, protein and fat contents of claw meat and hepatopancreas. The yields of crab claw meat and hepatopancreas were only 24.9–26.1% and 8.8–9.2%, respectively. Crude protein (NX6.25) and crude fat contents of crab claw meat were 17.8–18.2% and 0.85–1%, respectively on a dry weight basis, while those of hepatopancreas were 13–14% and 21.8–22.7%, respectively. These values are in good agreement with those published for green crab (Skonberg & Perkins, 2002), but lower than those reported by Naczk et al. (2004) for green crab from Nova Scotia, Canada.

As shown in Table 3, the proportion of neutral lipid (SE, TG, CHL, DG, FFA and MG) and polar lipids (PC, PE, PI and PS) were determined. Comparable amounts were obtained in the hepatopancreas and in the claw meat.

The fatty acid composition of green crab harvested from four sites was determined. No statistically significant difference of the fatty acid composition was found between crabs collected from the four harvesting sites. The fatty acid profiles were significantly different between crab claw meat (Table 4), and hepatopancreas (Table 5). The percentage of total saturated fatty acids was higher in the hepatopancreas than in the claw meat (25.15–26.24% and 22.5–23.49%, respectively). The main saturated fatty acids were palmitic (16:0) (11.5–12.45% and 11.0–11.5% in the hepatopancreas and in the claw meat, respectively) and stearic (18:0) acids

Table 1
Proximate composition (%) of crab hepatopancreas

Location	Yield (%)	Moisture (%)	Protein (%)	Fat (%)
Site # 1	9 ± 0.09	65 ± 0.5	13.5 ± 0.7	22 ± 0.5
Site # 2	8.8 ± 0.1	64.8 ± 0.2	13 ± 0.6	21.9 ± 0.5
Site # 3	9.1 ± 0.05	65.1 ± 0.4	13.9 ± 0.8	22.7 ± 0.6
Site # 4	9.2 ± 0.02	65 ± 0.9	14 ± 0.5	21.8 ± 0.5

Yield: results were mean values from 10 crabs; others were mean ($n = 3$) from the same sample. Values in the same column not sharing the same superscript are significantly different ($p < 0.05$), based on ANOVA.

Table 2
Proximate composition (%) of crab claw meat

Location	Yield (%)	Moisture (%)	Protein (%)	Fat (%)
Site # 1	25.3 ± 0.5	80 ± 0.9	18 ± 0.5	1 ± 0.02
Site # 2	24.9 ± 0.2	81 ± 0.3	17.8 ± 0.9	0.85 ± 0.05
Site # 3	26.1 ± 0.3	79 ± 0.9	18.1 ± 0.4	0.95 ± 0.01
Site # 4	25.6 ± 0.3	81 ± 0.6	18.2 ± 0.2	0.99 ± 0.02

Yield: results were mean values from 10 crabs; others were mean ($n = 3$) from the same sample. Values in the same column not sharing the same superscript are significantly different ($p < 0.05$), based on ANOVA.

Table 3
Proportion of neutral lipid and polar lipid of claw meat and hepatopancreas

Location	Claw meat composition (% of total lipid)		Hepatopancreas composition (% of total lipid)	
	Neutral lipid	Polar lipid	Neutral lipid	Polar lipid
Site # 1	54 ± 2	46 ± 2	41 ± 2	59 ± 1
Site # 2	51 ± 3	49 ± 1	40 ± 1	60 ± 2
Site # 3	56 ± 2	44 ± 2	43 ± 1	57 ± 2
Site # 4	55 ± 3	45 ± 1	40 ± 2	59 ± 1

Yield: results were mean values from 10 crabs; others were mean ($n = 3$) from the same sample. Values in the same column not sharing the same superscript are significantly different ($p < 0.05$), based on ANOVA.

Table 4
Fatty acid composition of crab claw meat (% of total fatty acid)

Fatty acid	Site # 1	Site # 2	Site # 3	Site # 4
C14:0	0.64 ± 0.02	0.66 ± 0.03	0.63 ± 0.02	0.67 ± 0.02
C15:0	0.84 ± 0.03	0.83 ± 0.02	0.9 ± 0.02	0.82 ± 0.03
C16:0	11.50 ± 0.02	11.2 ± 0.1	11.0 ± 0.03	11.3 ± 0.02
C17:0	2.3 ± 0.02	2.5 ± 0.1	2.5 ± 0.02	2.7 ± 0.02
C18:0	7.25 ± 0.3	7.0 ± 0.5	7.0 ± 0.5	7.3 ± 0.3
C20:0	0.5 ± 0.02	0.9 ± 0.3	0.55 ± 0.01	0.7 ± 0.02
C16:1n-7	3.9 ± 0.1	4.2 ± 0.2	4.1 ± 0.2	4.0 ± 0.1
C18:1n-9	11.4 ± 0.2	10.9 ± 0.4	11.0 ± 0.5	11.2 ± 0.2
C18:1n-7	4.2 ± 0.1	4.5 ± 0.2	4.4 ± 0.1	4.5 ± 0.1
C20:1n-9	1.4 ± 0.1	1.7 ± 0.07	1.2 ± 0.1	1.5 ± 0.05
C20:1n-7	0.96 ± 0.05	1.1 ± 0.05	0.75 ± 0.07	0.85 ± 0.01
C22:1n-9	0.86 ± 0.01	0.95 ± 0.05	0.7 ± 0.02	0.9 ± 0.2
C22:1n-7	0.46 ± 0.2	0.75 ± 0.02	0.6 ± 0.1	0.8 ± 0.3
C18:2n-6	1.9 ± 0.3	2.1 ± 0.1	2.0 ± 0.05	2.5 ± 0.03
C20:2n-6	0.63 ± 0.03	0.5 ± 0.1	0.5 ± 0.02	0.7 ± 0.03
C18:3n-3	0.8 ± 0.03	1.0 ± 0.1	0.7 ± 0.03	1.1 ± 0.5
C20:4n-6	11.8 ± 0.5	10.5 ± 0.5	11.2 ± 0.5	10.9 ± 0.05
C22:4n-6	0.74 ± 0.05	0.85 ± 0.05	0.9 ± 0.02	0.75 ± 0.5
C20:5n-3	9.3 ± 0.5	8.9 ± 0.4	9.2 ± 0.5	9.0 ± 0.5
C22:5n-3	1.36 ± 0.1	1.77 ± 0.1	1.54 ± 0.1	1.81 ± 0.1
C22:6n-3	10.8 ± 0.5	10.5 ± 0.9	10.1 ± 0.5	10.0 ± 0.3
Others	16.49 ± 0.3	16.78 ± 0.5	18.26 ± 0.3	16.85 ± 0.5
Saturated	23.03 ± 0.7	23.09 ± 0.5	22.58 ± 0.5	23.49 ± 0.3
Monounsaturated	23.18 ± 0.5	24.01 ± 0.7	22.75 ± 0.5	22.9 ± 0.3
Polyunsaturated	37.33 ± 0.9	36.12 ± 0.9	36.14 ± 0.7	36.76 ± 0.5
n-6	15.07 ± 0.5	13.95 ± 0.2	14.6 ± 0.2	14.85 ± 0.5
n-3	22.26 ± 0.2	22.17 ± 0.2	21.54 ± 0.5	21.91 ± 0.5

Table 5
Fatty acid composition of crab hepatopancreas (% of total fatty acid)

Fatty acid	Site # 1	Site # 2	Site # 3	Site # 4
C14:0	0.77 ± 0.02	0.76 ± 0.03	0.75 ± 0.02	0.77 ± 0.02
C15:0	0.92 ± 0.03	0.93 ± 0.02	1.0 ± 0.02	0.8 ± 0.03
C16:0	12.45 ± 0.02	12.25 ± 0.1	11.5 ± 0.03	12.1 ± 0.02
C17:0	2.7 ± 0.02	3.1 ± 0.1	2.9 ± 0.02	2.5 ± 0.02
C18:0	8.1 ± 0.3	8.0 ± 0.5	7.8 ± 0.5	8.3 ± 0.3
C20:0	0.9 ± 0.02	1.2 ± 0.3	1.2 ± 0.01	1.0 ± 0.02
C16:1n-7	3.7 ± 0.1	3.75 ± 0.2	4.0 ± 0.2	3.9 ± 0.1
C18:1n-9	12.7 ± 0.2	12.65 ± 0.4	12.5 ± 0.5	12.5 ± 0.2
C18:1n-7	3.92 ± 0.1	4.2 ± 0.2	3.85 ± 0.1	3.65 ± 0.1
C20:1n-9	1.1 ± 0.1	1.2 ± 0.07	1.0 ± 0.1	1.0 ± 0.05
C20:1n-7	0.8 ± 0.05	1.0 ± 0.05	0.95 ± 0.07	0.95 ± 0.01
C22:1n-9	0.8 ± 0.01	1.0 ± 0.05	0.75 ± 0.02	0.75 ± 0.2
C22:1n-7	0.26 ± 0.2	0.25 ± 0.02	0.35 ± 0.1	0.35 ± 0.3
C18:2n-6	2.1 ± 0.3	2.3 ± 0.1	2.0 ± 0.05	2.2 ± 0.03
C20:2n-6	0.9 ± 0.03	1.1 ± 0.1	1.0 ± 0.02	1.1 ± 0.03
C18:3n-3	0.9 ± 0.03	1.2 ± 0.1	1.2 ± 0.03	0.8 ± 0.5
C20:4n-6	13.3 ± 0.5	13.5 ± 0.5	13.2 ± 0.5	13.0 ± 0.05
C22:4n-6	0.61 ± 0.05	0.82 ± 0.05	0.5 ± 0.02	0.54 ± 0.5
C20:5n-3	10.9 ± 0.5	10.95 ± 0.4	10.7 ± 0.5	11.1 ± 0.5
C22:5n-3	1.22 ± 0.1	1.66 ± 0.1	1.74 ± 0.1	1.43 ± 0.1
C22:6n-3	10.4 ± 0.5	11.4 ± 0.9	10.9 ± 0.5	10.1 ± 0.3
Others	11.35 ± 0.3	7.58 ± 0.5	11.01 ± 0.3	12.96 ± 0.5
Saturated	25.84 ± 0.7	26.24 ± 0.5	25.15 ± 0.5	25.47 ± 0.3
Monounsaturated	23.28 ± 0.5	24.05 ± 0.7	23.4 ± 0.5	23.1 ± 0.3
Polyunsaturated	40.33 ± 0.9	42.93 ± 0.9	41.24 ± 0.7	40.27 ± 0.5
n-6	16.91 ± 0.5	17.72 ± 0.2	16.7 ± 0.2	16.84 ± 0.5
n-3	23.42 ± 0.5	25.21 ± 0.5	24.54 ± 0.2	23.43 ± 0.5

Results are mean values of duplicate injection of two samples. SDs from means did not exceed 1% of absolute values.

(7.8–8.3% and 7.0–7.3%, respectively). Meanwhile, oleic acid (18:1) was the dominant monounsaturated fatty acid (16.5–16.85% and 15.4–15.7% in the claw meat and hepatopancreas, respectively. These values are similar to those reported by Naczk et al. (2004) for green crab (*Carcinus maenas*).

The fatty acid profile of green crab lipids was dominated by polyunsaturated fatty acids (PUFA) (40.27–42.93% and 36.12–37.33% in hepatopancreas and in the claw meat, respectively). The total n-6 fatty acids were 16.7–17.62% and 13.95–15.07% in the hepatopancreas and in the claw meat, respectively. The major n-6 PUFA was arachidonic acid (20: 4n-6) which its content was higher in the hepatopancreas (13–13.5%) than in the claw meat (10.5–11.8%). Similar contents of crab meat arachidonic acid were reported by Naczk et al. (2004) for green crab (*C. maenas*), whereas higher values were reported by King, Dorset, and Monsen (1990) for Dungeness crab. The major n-3 PUFA were EPA (20:5n-3) and DHA (22:6n-3) and the ratio of EPA to DHA present an average of 1.0. These values are lower than those reported by Naczk et al. (2004) for Nova Scotia green crab, but similar to those reported by Skonberg and Perkins (2002) for green crab caught in the Gulf of Maine. The observed difference in the EPA to DHA ratio in this study compared to those reported by Naczk et al. (2004) may be due to the existing differences in the diet of crab at the harvesting sites. The n-3 fatty acids accounted for (23.42–25.21% and 21.54–22.26% in the hepatopancreas and in the claw meat, respectively) of the total and (58–59% and 59–61% in the hepatopancreas and in the claw meat, respectively) of all PUFA. The total contents of n-3 fatty acids were somewhat lower than those reported in the literature for Nova Scotia green crab (Naczk et al., 2004).

PUFA are beneficial for the human health, since their intake would lower the risk of developing artherosclerosis and cholesterol accumulation in the blood. A comparison of the free fatty acids contents of the green crab with those reported for other crab species shows few differences (King et al., 1990; Naczk et al., 2004). These differences are apparently associated with variations between species, nutrient composition of the diet, the surrounding medium, the season and other factors characteristics of the crab physiology.

We wanted to check wether the human pancreatic lipase present in the pancreatic juice of a healthy donor was active on an emulsion of crab's lipids. This juice contains lipase and colipase, as well as a physiological concentration of bile salts. Its activity on an olive oil emulsion was found to be 60 U/ml. A significant activity of the pancreatic lipase present in this juice was found (30 U/ml) when using meat or hepatopancreas crab lipids emulsion as substrate. The hydrolysis level tested *in vitro* is only a qualitative indication of the efficient activity of the human pancreatic juice on the crab lipids, as the *in vivo* activity of the same juice on a test liquid or solid meal was found to be significantly different from that measured in vitro (Carrière et al., 2000).

4. Conclusion

It can be concluded that claw meat and hepatopancreas of green crab from Tunisian coasts are good sources of proteins and PUFAs. The results clearly indicate that Tunisian green crab is a nutritious food. A preparation of green crab lipids was efficiently hydrolysed by human pancreatic juice *in vitro*, suggesting a good digestibility of these lipids *in vivo*.

Acknowledgements

We acknowledge Mr. J. Makni "Office National de l'huile, Sfax" for his technical assistance. We thank Drs. Alain De Caro, Frederic Carriere and Rene Laugier for their generous gift of the Human pancreatic juice. This work is part of a doctoral thesis by Slim Cherif. This work received financial support from "Ministère de l'enseignement supérieur et de la recherche et de la technologie" granted to the Laboratoire de Biochimie et de Génie Enzymatique des Lipases.

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