



Lipids and of fatty acids of edible crabs of the north-western Pacific

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ABSTRACT

Analyses of lipids and fatty acids in muscles and hepatopancreas of five commercially exploited crabs inhabiting the Sea of Japan and the Okhotsk Sea, namely *Paralithodes camtschaticus*, *Paralithodes platypus*, *Chionoecetes opilio*, *Chionoecetes angulatus* and *Chionoecetes japonicus*, have been carried out. The total lipid level (TL) in muscles ranged from 0.53% of wet weight (ww) to 1.57% of ww and the amount of phospholipids exceeded that of triglycerides. The TL contents in the hepatopancreas of all crabs were higher than in muscles and varied between 10.2% ww in *C. angulatus* and 19.8% ww in *P. platypus*, the major class of lipids being triglycerides. The main polar lipids in the hepatopancreas and muscles were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Among polyunsaturated fatty acids (PUFAs), the *n*-3 fatty acids have dominated; 16:0, 18:1*n*-9, 20:5*n*-3 and 22:6*n*-3 were the main fatty acids contained in the tissues studied. In all the crabs, excluding *C. angulatus*, the PUFAs *n*-3/*n*-6 ratio in muscles varied between 7.02 and 10.3 while, in the hepatopancreas, the ratio varied between 4.00 and 6.62.

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1. Introduction

Crabs are a large group of invertebrates and, due to the high palatability of their meat, they are a focus of commercial fisheries. In the Sea of Japan and the Okhotsk Sea, the crab commercial catch is generally based on five species of the genus *Paralithodes* or *Chionoecetes*. For a long time, king crabs of the genus *Paralithodes* – *P. camtschaticus* and *P. platypus* – were the major commercial species, but collapse of natural populations made the species of genus *Chionoecetes* more attractive for fishing.

The composition of proteins, amino acids, sterols, total lipids and fatty acids of the most commercially significant crabs can be found in earlier publications (Gates & Parker, 1992; Krzynowek, Wiggin, & Donahue, 1982). The meat of *Eriocheir sinensis*, for instance, contains all amino acids essential for humans (Chen, Zhang, & Shrestha, 2007). At the same time, the nutritive value of crabs is primarily determined by lipids, fatty acid *n*-3 PUFAs. Despite the fact that crabs form a significant part of seafood, the information on lipids and fatty acids of crabs is scattered and cannot be considered complete (Celik et al., 2004; Naczki, Williams, Brennan, Liyanpathirana, & Shahidi, 2004). Furthermore, within recent years, *Carcinus maenas* (Skonberg & Perkins, 2002) and deep-sea species of *C. japonicus* and *C. angulatus* (Somerton & Donaldson, 1995), not previously of any commercial importance, have been introduced into the crab business.

The role of *n*-3 PUFAs in human nutrition and prevention of many immunodeficient and cardiovascular diseases is well-known.

C-20 *n*-3 and *n*-6 fatty acids are prominent among PUFAs since they are precursors of the hormone-like compounds known as oxylipins. These compounds control many biochemical and physiological processes in the human organism (Holman, 1997). The synthesis of *n*-3 *C*-20 PUFAs occurs in aquatic organisms, mainly in algae, and it then further proceeds through food chains to a higher trophic level, including humans.

Seafood is unique in having easily digestible proteins and essential *n*-3 PUFAs in its composition (Guddings & Hill, 1975). The enhanced catch and consumption of crabs, worldwide, require more detailed information on the lipid and fatty acids composition. Since the northern Pacific is considered to be one of the major world crab catching areas, the composition of lipids and fatty acids of the main commercial species was studied.

2. Materials and methods

The animals were trapped during summer–autumn, 2005. *P. camtschaticus* was harvested in Tatar Strait (Sea of Japan), *P. platypus* off Iona Island (Okhotsk Sea), and *C. opilio*, *C. angulatus* and *C. japonicus* off the north-eastern coast of Sakhalin Island (Okhotsk Sea). Consistent with fishery regulations, the commercial catching of female crabs is prohibited, so for the present analysis we used leg meat and the hepatopancreas of male crabs. The five animals were prepared just after the catch and, in each case, we took equal amounts (20–30 g) of specific tissues. The tissue specimens of each species to be analyzed were kept at –20 °C.

To examine lipids, the pooled tissues were homogenized and aliquots (~10–20 g) were extracted by chloroform–methanol (2:1 v/v). Total lipids were separated into polar and neutral fractions

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by silica-gel column chromatography. The neutral lipids were eluted with five column volumes of chloroform while the polar lipids were then eluted with 10 column volumes of methanol. The phospholipids were separated by two-dimensional thin-layer chromatography (TLC) on silica-gel and identified by the use standards and specific sprays. Quantitative analysis of separated phospholipids was performed according Vaskovsky, Kostetsky, and Vasendin (1975).

Fatty acid methyl esters (FAME) were obtained by gradual processing of total lipids with 1% solutions of MeONa/MeOH and 5% HCl/MeOH, and purified by preparative TLC in benzene as solvent. The analysis of FAME was done on a Shimadzu GC-17A chromatograph (Kyoto, Japan) with a flame ionization detector on a Supelcowax 10 (Supelco, Bellefonte, PA) capillary column (30 m × 0.25 mm i.d.) at 210 °C. Helium was used as a carrier gas. FAME were identified by comparison with authentic standards and using a table of ECL values (Stransky, Jursik, & Vitek, 1997).

A Statistica 5.5 computer package was used for data analysis. A Student's *t*-test (independent variables) was used to check for differences between two means, at 95% significance level. Principal component analysis was used to identify interspecific differences in fatty acids composition of muscles. We analyzed the following fatty acids, which had maximal concentrations: 16:0, 16:1*n*-7, 18:0, 18:1*n*-9, 18:1*n*-7, sum of the 20:1 monoenoic, 20:2*n*-6, 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3.

3. Results and discussion

The results of the study revealed that the average values of total lipid content in muscle tissue and the hepatopancreas differs markedly (Table 1), the result being significant at the 0.01 level of confidence. The amount of TL in the species under investigation varied (in muscles) between 0.53% ww in *C. japonicus* and 1.57% ww in *C. opilio*. These values are close to those previously published for the green crab, *C. maenas* (Skonberg & Perkins, 2002), but higher than those in the muscles of *C. opilio* (Addison, Ackman, & Hingley, 1972) and blue crab, *Callinectes sapidus* (Gates & Parker, 1992; Tsai, Chen, & Tsai, 1984). According to the data of various authors, TL in leg muscles of *P. camtschaticus* ranged from 0.5% ww (Sidwell, Foncannon, Moore, & Bonnet, 1974) to 2.6% ww (Ottwell, Bellairs, & Sweat, 1984). In this study, TL content for *P. camtschaticus* was 0.61% ww. Such intraspecific differences in lipid content of muscles can be accounted for by different inhabiting conditions or moulting stages of crabs. It was shown that, in *P. camtschaticus*, for instance, living off the coast of Alaska, the total muscle lipids made up 2% ww (Krzczykowski, Tenney, & Kelley, 1971) whereas this value was not higher than 0.6% ww (Konosu, Yamagushi, & Hayashi, 1978) in crabs from the southern part of

the Sea of Okhotsk. Nevertheless, in the report of presented by Vanconcelos and Braz (2001) there is a resume of the fat content in leg muscles of nine species of commercial crabs approximately corresponding to the data received.

Most of the muscle lipids consisted of phospholipids, except for *C. japonicus* and *P. platypus* with prevailing triglycerides (Table 1). The amount of total polar lipids in muscles was low and varied between 0.23% ww in *C. japonicus* and 1.18% ww in *C. opilio*. Our data were close to data obtained for the muscles of *E. sinensis* – 0.85% ww, *C. maenas* – 0.74% ww (Chapelle, 1977) and *C. opilio* – 0.75% ww (Addison et al., 1972).

Neutral lipid content in the hepatopancreas of crabs, genus Paralithodes, on the average, was higher than that in crabs of the genus Chionoecetes. However, significant fluctuations in the neutral lipid content were registered for the genus Chionoecetes, from 9.49% ww to 14.0% ww, and for the genus Paralithodes, from 11.4% ww to 18.4% ww, respectively. Such variability may be related to various factors, namely, food, sex, habitation and moulting stages of crabs (Lautier & Lagarrigue, 1986; Styrisshave & Andersen, 2000; Tsai et al., 1984).

It is evident that the abundance or lack of food and its quality may change the amount of triglycerides in the hepatopancreas rather quickly and, as a result, may influence the polar–neutral lipid ratio (Harrison, 1990). Crab hepatopancreas is rarely used as food and probably this is the main reason for scarce data on the lipid composition of this organ. In viscera of *C. opilio*, for instance, harvested off the Atlantic Provinces coast of Canada, the lipid content accounted for 13.6% of ww (Addison et al., 1972) and this agreed with our results for this species. According to the data obtained by Chapelle (1977), the total lipid content in the hepatopancreas of the Chinese crab *E. sinensis* and green crab, *C. maenas*, was much lower than that in the animals studied in the present paper.

The distribution of phospholipids in tissues, according to classes, showed that PC and PE were the major components of polar lipids. The total phospholipid content in muscles and hepatopancreas of crabs of the genus Paralithodes was higher than that of the genus Chionoecetes. Some samples of lipids had a significant level of lysoderivatives PC and PE which is likely related to a high activity of lipolytic enzymes exhibiting hydrolytic activity at temperatures lower than 0 °C (Hardy, 1980). It should be noted that the evidence available in the literature, concerning complete phospholipid composition, is confined to Chapelle's work (1977) in which the lipid compositions of *E. sinensis* and *C. maenas* were studied. In the literature, there is insufficient information on phospholipids of the species studied here, except for an early publication on some phospholipids of *C. opilio* muscles investigated by Addison et al. (1972) and De Koning (1970).

The distribution of fatty acids, in TL extracted from muscles and the hepatopancreas, is presented in Table 2. The lipids of these tis-

Table 1
Lipid composition of tissues of crabs of genus Paralithodes and Chionoecetes.

		TL	PL	NL	PC	LPC	PE	LPE	PS	PI	SM	PA
		g/100 g of wet weight			% of total phospholipids							
<i>P. camtschaticus</i>	Hepatopancreas	13.6	2.21	11.4	72.8	–	19.3	–	2.20	3.90	1.80	–
	Muscle	0.61	0.37	0.24	74.1	0.20	13.8	–	1.90	3.30	4.80	1.90
<i>P. platypus</i>	Hepatopancreas	19.8	1.47	18.4	54.2	21.10	2.70	16.8	1.60	3.50	tr	tr
	Muscle	0.60	0.34	0.26	73.6	–	18.4	–	1.10	1.50	5.40	–
<i>C. opilio</i>	Hepatopancreas	13.0	0.52	12.5	62.0	4.30	18.2	6.40	2.10	3.70	3.20	–
	Muscle	1.57	1.18	0.39	62.6	6.30	17.8	6.60	1.30	3.30	2.00	tr
<i>C. japonicus</i>	Hepatopancreas	15.8	1.80	14.0	58.6	–	32.2	–	3.34	3.35	2.30	–
	Muscle	0.53	0.31	0.23	59.0	5.40	21.6	6.70	0.80	2.00	3.50	–
<i>C. angulatus</i>	Hepatopancreas	10.2	0.75	9.49	67.7	1.40	19.3	tr	2.10	1.70	6.80	tr
	Muscle	0.96	0.74	0.22	61.3	2.50	23.6	1.20	4.20	4.10	2.80	–

TL, total lipids; PL, polar lipids; NL, neutral lipids; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; LPC, lysophosphatidyl choline; LPE, lysophosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidyl inositol; SM, sphingomyeline; PA, phosphatidic acid.

Table 2Fatty acid (FA) composition of tissues of crabs of genus *Paralithodes* and *Chionoecetes* (% of total FA).

Fatty acid	<i>Paralithodes</i>				<i>Chionoecetes</i>					
	<i>Camtschaticus</i>		<i>Platipus</i>		<i>Opilio</i>		<i>Japonicus</i>		<i>Angulatus</i>	
	Meat	Hepatopancreas	Meat	Hepatopancreas	Meat	Hepatopancreas	Meat	Hepatopancreas	Meat	Hepatopancreas
14:0	0.93	1.64	1.63	2.43	0.24	2.50	0.61	2.42	0.60	1.60
15:0ai	–	2.29	–	–	–	0.33	–	0.17	–	–
15:0	0.55	0.98	0.41	0.37	0.16	0.36	0.24	0.32	0.35	0.22
16:0-i	–	1.48	–	–	–	0.23	–	0.20	–	0.23
16:0	11.9	8.04	12.7	9.03	12.8	11.4	14.4	12.9	10.3	9.96
16:1 <i>n</i> -7	3.01	4.58	3.27	6.44	2.64	10.5	1.18	6.21	7.11	4.20
17:0-i	–	0.53	–	0.39	–	0.63	–	0.21	–	0.41
17:0ai	–	1.02	–	0.23	–	0.81	–	0.33	–	0.36
16:2 <i>n</i> -6	0.20	0.73	0.97	0.38	0.17	1.22	0.12	0.53	0.46	1.01
17:0	0.53	–	0.38	–	0.51	–	0.44	–	0.53	–
17:1 <i>n</i> -7	0.54	0.57	0.37	0.61	0.29	0.61	0.15	0.35	0.64	0.27
16:3 <i>n</i> -3	0.25	0.63	0.51	0.34	0.59	0.32	0.66	0.73	0.61	0.30
16:4 <i>n</i> -3	–	–	–	–	–	–	–	0.93	–	–
18:0	4.21	4.32	2.80	1.22	2.37	2.03	2.57	1.80	4.21	1.65
18:1 <i>n</i> -11	–	0.68	–	–	–	–	–	–	–	–
18:1 <i>n</i> -9	11.7	8.32	13.3	22.3	9.29	16.2	11.9	22.1	16.3	14.3
18:1 <i>n</i> -7	7.93	8.40	6.09	4.78	8.95	7.81	8.2	7.58	8.84	5.94
18:1 <i>n</i> -5	0.50	0.51	1.30	1.10	1.08	0.65	0.85	0.72	1.27	0.91
18:2 <i>n</i> -6	0.89	0.87	0.75	0.83	0.69	0.67	0.74	0.88	0.93	0.75
18:2 <i>n</i> -4	–	0.56	–	–	–	0.35	–	0.23	–	0.21
18:3 <i>n</i> -6	–	0.24	–	–	–	–	–	0.34	–	0.20
18:3 <i>n</i> -3	–	0.18	0.17	0.26	0.15	0.17	0.51	0.41	–	0.19
18:4 <i>n</i> -3	–	0.52	–	0.26	–	0.57	–	0.44	–	0.23
20:1 <i>n</i> -11	2.34	8.35	3.30	9.80	1.60	8.00	0.88	3.55	5.10	14.7
20:1 <i>n</i> -9	–	–	–	–	–	–	–	5.36	–	–
Σ 20:2 NMI ^a	0.17	0.62	–	0.70	–	0.60	1.58	1.52	0.24	–
20:2 <i>n</i> -6	0.49	0.85	0.53	0.60	0.51	0.62	0.64	0.28	2.01	0.62
20:4 <i>n</i> -6	4.13	3.59	2.33	2.02	3.25	1.61	3.76	1.37	10.2	2.10
20:4 <i>n</i> -3	–	0.29	–	0.56	–	0.28	–	0.21	–	–
20:5 <i>n</i> -3	31.1	17.8	29.0	13.5	31.8	12.8	22.4	7.36	13.5	8.11
22:1 <i>n</i> -11	0.20	1.97	1.10	2.10	0.70	2.80	0.66	–	1.70	9.60
22:1 <i>n</i> -9	0.65	–	–	–	–	–	1.03	3.72	–	–
21:5 <i>n</i> -3	–	0.59	–	0.26	–	0.51	–	0.10	–	0.20
22:4 <i>n</i> -6	–	1.04	–	0.21	–	0.73	–	0.21	–	0.58
22:5 <i>n</i> -6	0.33	0.51	0.29	0.41	0.36	0.28	0.41	0.21	0.34	0.28
22:5 <i>n</i> -3	0.84	1.22	0.60	1.18	0.99	1.5	0.38	1.49	1.09	1.42
22:6 <i>n</i> -3	9.78	8.18	13.9	10.7	17.3	6.57	19.2	9.32	5.92	11.4
24:6 <i>n</i> -3	0.43	0.96	0.57	2.40	0.23	0.15	–	1.35	–	–
Others	6.46	6.96	3.76	4.60	3.36	6.19	6.47	4.15	7.70	8.08
Σ SAFA ^b	18.1	20.3	17.9	13.7	16.1	18.3	18.3	18.4	16.0	14.4
Σ MUFA ^c	26.8	33.4	28.7	47.1	24.6	46.6	24.8	49.6	41.0	49.9
Σ PUFA ^d	48.6	39.1	49.6	34.6	56.0	29.0	50.5	27.6	35.3	27.4
Σ <i>n</i> -3 PUFA	42.4	30.4	44.8	29.5	51.1	22.9	43.2	22.3	21.2	21.8
Σ <i>n</i> -6 PUFA	6.04	7.59	4.87	4.45	4.98	5.13	5.67	3.48	13.9	5.34
<i>n</i> -3/ <i>n</i> -6	7.02	4.00	9.19	6.62	10.3	4.47	7.62	6.42	1.52	4.08

^a NMI, non-methylene-interrupted fatty acids 20:2Δ5,13 and 20:2Δ7,15.^b SAFA saturated fatty acids.^c MUFA monounsaturated fatty acids.^d PUFA polyunsaturated fatty acids.

sues were relatively rich in PUFAs and are typical of marine organisms (Joseph, 1989). The main saturated fatty acids were palmitic (16:0) and stearic (18:0), while oleic acid (18:1*n*-9) prevailed among monounsaturated acids.

In the saturated acid content in lipids of the hepatopancreas of the crabs studied, except for *C. angulatus*, no significant differences were revealed between the species. However, the amount of monounsaturated fatty acids in the hepatopancreas of all crabs was higher than that in muscle lipids, except for the contents of the 16:1*n*-7 and 18:1 isomers in muscles of *C. angulatus*, which were higher than those in the hepatopancreas. As mentioned above, the higher amount of total monoenoic acids in lipids of the hepatopancreas than in muscles is characteristic of lipid composition in Decapoda (Celik et al., 2004; Harrison, 1990). The highest relative difference between hepatopancreas and muscles was registered for C-20 monoenes and this, most probably, is connected with food sources rich in these isomers and with biosynthesis, *in vivo*, occurring in the hepatopancreas.

High PUFAs level in the total lipids, in all the crabs under study, is not unexpected. It is common knowledge that the lipids of marine invertebrates are abundant in PUFAs, this being typical of marine crustacea (Joseph, 1989). Their distribution in crab organs, however, is of a peculiar nature.

In muscle lipids the PUFAs were the principal class of fatty acids and varied between 35.3% (*C. angulatus*) and 56.0% (*C. opilio*) while, in the hepatopancreas, their contents were 27.4% (*C. angulatus*) and 39.1% (*P. camtschaticus*) (Table 2). The main PUFAs in lipids of both organs were 20:5*n*-3 and 22:6*n*-3 and in muscles they constituted the major part of the sum of total acids. These values were similar to those given by Krzynowek et al. (1982) for three species of commercial crabs and for total lipids of viscera and muscles of *C. opilio* (Addison et al., 1972). In the lipids of green crab *C. maenas*, however, 20:5*n*-3 and 22:6*n*-3 contents were much lower (Skonberg & Perkins, 2002). Distribution of *n*-6 acids and, in particular, of the main arachidonic acid (20:4*n*-6), in all lipids of muscles and the hepatopancreas, is of interest. The 20:4*n*-6 content in muscle

lipids, in all crabs, was higher than that in the hepatopancreas (Table 2) but the total amount did not exceed 4.13% (*P. camtschaticus*). The high level 20:4n-6 in TL of *C. angulatus* muscles (10.2%) and relatively low level of this acid in lipids of the hepatopancreas (2.1%) deserve attention. The distinctive feature of the distribution of n-6 fatty acids in the hepatopancreas was the noticeable amounts of 22:4n-6 and 22:5n-6 acids. The distribution of these acids by organs was specific: 22:4n-6 was found only in the hepatopancreas whereas 22:5n-6 was present both in muscles and the hepatopancreas. These acids can form, as a result of retroconversion, out of tetracosapolyenoic acids, 24:5n-6 and 24:6n-3 (Sprecher, 2000). They were revealed in marked amounts in some benthic invertebrates, namely Crinoidea and Ophiuroidea (Takagi, Kaneniva, & Itabashi, 1986) and Coelenterates (Vysotskii & Sveta-shev, 1991). Crabs are considered active predators and their diet consists mostly of benthic invertebrates, echinoderms and coelenterates being the main food, especially for deep-sea crabs (Wieczorek & Hooper, 1995). The best evidence for crab's feeding on these benthic invertebrates is the 24:6n-3 acid content, detected only in the hepatopancreas (Table 2).

The importance of a balanced PUFAs intake has been recognized by health organizations throughout the world over the past decade, and there is now a consensus that PUFAs should form at least 3%, and preferably 8–23%, of the total lipid intake, and that the n-3/n-6 ratio should ideally lie between 1:4 and 1:10 (Gill & Valivety, 1997).

Western diets are considered 'relatively deficient' in omega-3 fatty acids, because they contain excessive amounts of omega-6 fatty acids (Holman, 1997). That is why fish oils and specific preparations based on them are used to compensate for the shortage of n-3 PUFAs in human diet (Shahidi & Wanasundra, 1998). Thus, a low cholesterol content of crab meat, the presence of all essential amino acids and high 20:5n-3 and 22:6n-3 contents characterise this product as important (Krzyonowek et al., 1982).

The hepatopancreas of decapod crustaceans, as a rule, is not used for food but a very high fraction of triglycerides in total lipid can be a source of both natural oil and preparations of essential n-3 PUFAs (Shahidi & Wanasundra, 1998). Furthermore, the hepatopancreas is rich in some proteolytic enzymes, including collagenases, widely applied in medicine and food technology.

The principle component analysis of fatty acids of the total muscle lipids showed that the crabs were divided into two groups according to their fatty acid composition (Fig. 1). The first group comprises *P. camtschaticus*, *P. platypus*, *C. opilio* and *C. japonicus*.

The second group is far removed from the first and includes *C. angulatus*. Such arrangement is due to much smaller 20:5n-3 and 22:6n-3 concentrations and higher 20:2n-6 concentrations which are part of the total lipid fatty acids in *C. angulatus* muscles.

The results obtained allow us to conclude that the lipid and fatty acid compositions confer nutritive value to all the crab species studied. The exception is *C. angulatus* that differs greatly from the other species in its composition of total lipid fatty acids.

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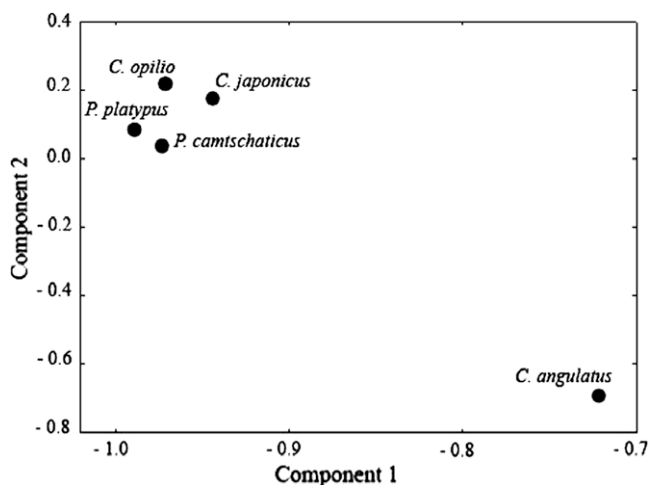


Fig. 1. Principal component analysis of fatty acids of the total lipids extracted from muscle of five species of crabs.

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