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Molecular Species Composition of Phosphatidylcholine Isolated from Chum Salmon Meat Oil

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Chum salmon (*Oncorhynchus keta*) meat oil contained high amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared to oils extracted from other tissues of the fish. EPA and DHA occupied more than 25% of the total fatty acids in chum salmon meat oil. The main lipid classes in the meat oil were triacylglycerides and phospholipids. The major fatty acids of the molecular species composition of phosphatidylcholine isolated from the meat oil were DHA and EPA. DHA and EPA were the major molecular species in the phosphatidylcholine of chum salmon meat oil, representing 44% and 17%, respectively.

Key words: Chum salmon meat, DHA, EPA, Phosphatidylcholine

Introduction

Seafoods from fish and marine invertebrates constitute globally important food sources. They are sources of proteins and lipids of high nutritional value in addition to being good sources of vitamins, minerals (Gonzalez et al., 2001), and other bioactive materials (Kim and Mendis. 2006). Fish are high in polyunsaturated fatty acids [n-3fatty acids, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3)], which play an important role in human health and nutrition. These n-3 polyunsaturated fatty acids (PUFAs) are effective at lowering blood pressure, reducing hyperlipidemia and arrhythmias, and preventing arterial thrombosis and cardiovascular disease; they are anti-inflammatory, anticancer, and anti-obesity molecules (Li et al., 2003).

In addition, the high levels of EPA and DHA in marine lipids imply the presence of a strong antioxidant system in marine animal tissues. Antioxidants such as tocopherols are regarded as extremely important for preventing oxidation of marine lipids, but other factors have also been reported. Totani and Hara et al. found that phospholipids (PLs) were important factors that inhibited lipid oxidation (Hara et al., 1992; Segawa et al., 1995; Takeuchi et al., 1997). The importance of PLs has also been reported in a comparative study on the oxidation of lipids from different sardine and mackerel tissues (Ohshima et al., 1993). In addition, PLs are known as synergists in combination with phenolic antioxidants such as tocopherols (Ishikwa et al., 1984; King et al., 1992). Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are the most abundant phospholipids of eukaryotic cells, comprising 25% and 50%, respectively, of cell phospholipid mass (White, 1973; Paltauf et al., 1992). We analyzed the molecular species composition of PC isolated from chum salmon meat oil.

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Materials and Methods

Materials

Chum salmon (*Oncorhynchus keta*), with body lengths of 57.7±2.3 cm and body weights of 1,502±58.5 g, were supplied by the Yangyang National fisheries Research & Development Institute located in Yangyang-gun, Korea, in November 2007. After the removal of roe, samples of the whole body, meat, viscera, and other tissues were taken from each fish. Samples were frozen at -70°C and then dried in a freeze-dryer.

Oil extraction

After putting 100 g of a vacuum freeze-dried sample in a 1,000 mL Erlenmeyer flask, we added 400 mL of 95% ethanol, which was four times greater (w/v) than the sample weight. Then, the sample was

sonicated for 30 min (PowerSonic 520; Hwashin, Seoul, Korea) at 30°C. After filtering with Whatman No. 2 filter paper, the extract was concentrated with a rotavator (R-200; Buchi, Flawil, Switzerland) and stored at -40°C.

Proximate analysis

Each chum salmon tissue sample was analyzed for its proximate composition (moisture content, crude protein, crude lipid, and crude ash) by the standard method of the Association of Official Analytical Chemists (AOAC, 1995). Carbohydrate content was calculated as the difference between 100% of the sample and the combined percentage of moisture, ash, crude protein, and total lipids.

Fatty acid composition

Fatty acid composition was analyzed by capillary gas chromatography (GC) after converting the fatty acyl groups in each lipid to their methyl esters by heating in a sealed tube at 90-100ºC for 1 h with 7% boron trifluoride in methanol under nitrogen. The GC analytical conditions are shown in Table 1.

Table 1. Condition of gaschromatography analysis

Oven	Setpoint-120°C, Max.-300°C Equilibration-3.00 min
Inlet	Mode-split, Total flow-75.6 mL Pressure- 9.52 psi, Split ratio-50:1 Split flow- 71.8 mL/MIN, Heater-250°C Gas saver- 20.0 mL/min. Gas-He
Column	HP-5 (5% Phenyl methyl Siloxane), Temp.- 325°C
Detector	Heater- 250°C, H_2 flow- 40 mL/min Air flow -450 mL/min Makeup flow-45.0, Gas-He

Isolation of phosphatidylcholine and phosphatidylethanolamine

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were further separated by silicic acid column chromatography using a chloroform/methanol solution as a developing solvent. Column chromatography separation was monitored by thin layer chromatography (TLC) using authentic standards, and was carried out on silica gel 60 GF plates (20×20 cm, 0.05 mm-thick layer; Merck, Darmstadt, Germany) that were developed with a chloroform/methanol/28% ammonia solution (65:35:5, v/v). Each of the PC and PE samples were minced and homogenized with chloroform/methanol (2:1, v/v) according to the method of Bligh and Dyer (1959).

Lipid class analyses

TLC was conducted on 0.25 mm silica gel plates (Merck) that were developed with a chloroform/methanol/28% ammonia solution (65:35:5, v/v/v). Lipid composition was determined quantitatively by a TLC-flame ionization detector (FID) on a Chromarod-S II using an Iatroscan TH-10 (Iatron, Tokyo, Japan). The sample solution was applied on each rod. The rods used for total lipid composition were developed 10 cm from the origin with chloroform/methanol/acetic acid (8:2:0.1, v/v/v). Also, the rods used for phospholipid composition were developed at the origin with chloroform/methanol/water (65:25:4, v/v/v). After the rods were developed, they were dried in a desiccator for a few minutes and scanned with the Iatroscan. The TLC-FID gas was hydrogen at a flow rate of 160 mL/min and the scan speed was 30 s.

Molecular species composition of phosphatidylcholine

PC and PE analyses used TLC manufactured with 0.5 mg/mL methanol. Samples (5 μL) were injected by HPLC into an AS3000 autosampler (Thermo Separation Production Products, San Jose, CA, USA), and a column (Gemini-5μ-c18-110A; Phenomenex, Torrance, CA, USA) was used. The column was kept at 20°C and had a flow solvent of MeOH/CAN/TEA (60:40:0.1, v/v) delivered at a flow rate of 0.5 mL/min by a P4000 pump (Thermo Finnigan, Hemel Hempstead, UK), an L-7610 degasser (Hitachi, Tokyo, Japan), a CTO-10AS column oven (Shimadzu, Otsu, Japan), and a LCQ MS (Finnigan).

Table 2. Proximate compositions of each tissue parts of vacuum freeze dried chum salmon

Body tissue sections	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude ash (%)
Whole body	6.10 ± .0.04	74.37 ± 1.41	8.89 ± 0.07	10.57 ± 0.21
Meat	4.34 ± 0.04	83.91 ± 0.58	5.67 ± 0.09	5.96 ± 0.30
Viscera	1.78 ± 0.04	81.95 ± 1.88	6.92 ± 0.29	7.70 ± 0.06
The others	3.45 ± 0.03	63.64 ± 0.15	12.58 ± 0.07	17.96 ± 0.75

Values are mean ± SD (n=9).

Results and Discussion

Proximate analysis

The proximate compositions of each vacuum freeze-dried chum salmon tissue sample are shown in Table 2. The lipid content of the vacuum freeze-dried meat was approximately 5.67±0.09%, and the protein content was approximately 83.91±0.58%.

Fatty acid composition

The percentages by weight (g/100 g) for total fatty acids in the oils of each tissue type are shown in Table 3. Saturated fatty acids comprised 33.05%, monoenoic fatty acids 38.14%, and polyenoic fatty acids 28.8%. The chum salmon fatty acid composition was similar to values report by Boyd et al. (1992), who examined the fatty acid composition of salmon meat oil. However, the values were different from the fatty acid composition of Coho salmon, for which saturated fatty acids comprised 24.43%, monoenoic fatty acids 43.79%, and polyenoic fatty acids 31.49% (Kim and Choi, 1993). The main fatty acids of chum salmon meat oil were palmitic acid (16:0), oleic acid (18:1n-9), EPA (20:5n-3), and DHA (22:6n-3). Oleic acid was the major monounsaturated fatty acid in chum salmon meat oil, representing 26.79%. Chum salmon meat oil had the highest EPA content at 20.71%. The EPA composition was similar to values reported by Moriya et al. (2007), who found that the EPA content of salmon roe and herring roe were 19.8% and 21.6%, respectively.

Table 3. Fatty acid composition of the oils extracted from each tissue parts of chum salmon

Fatty acid	Composition (%)			
	Whole body oil	Meat oil	Viscera oil	The others oil
4:0	1.86	0.97	2.11	1.89
12:0	0.43	0.07	0.12	0.23
14:0	5.38	3.43	3.73	7.22
15:0	0.57	0.39	0.43	0.66
16:0	19.30	16.64	22.46	19.53
17:0	0.32	0.40	0.50	0.55
18:0	5.07	3.83	6.52	4.57
20:0	0.12	0.31	-	0.24
21:0	-	-	-	-
22:0	-	-	-	-
23:0	-	0.11	-	-
24:0	-	-	-	-
Saturated	33.05	26.14	35.87	34.89
16:1	5.37	3.60	5.31	5.98
17:1	-	-	-	-
18:1T	1.20	0.93	1.43	1.21
18:1C	26.43	20.38	27.13	26.79
20:1	2.93	2.52	1.49	3.30
22:1	2.21	2.02	0.34	2.87
24:1	-	0.95	-	0.10
Monoenoic	38.14	30.39	35.69	40.26
18:2T	-	0.17	-	0.13
18:2C	1.95	2.93	1.34	1.74
18:3ω6	6.29	6.77	1.67	8.08
18:3ω3	1.73	1.77	1.01	1.85
20:2	0.57	0.83	0.48	0.86
20:3ω6	0.09	0.44	-	-
20:3ω3	0.09	0.41	-	0.22
20:4	0.81	1.00	1.77	0.58
22:2	0.00	0.09	-	0.15
20:5ω6	5.53	8.35	9.68	4.01
22:6ω3	11.74	20.71	12.48	7.22
Polyenoic	28.80	43.47	28.44	24.85

Values are mean±SD (n=9).

Lipid class analysis and molecular species composition

The lipid class composition of chum salmon meat oil is shown in Table 4. The triglyceride composition in the total lipids was 50.3±19.1%. For comparison, Moriya et al. (2007) reported that the triglyceride content of salmon roe was 71.8%. Also, as is shown in Table 4, the PE and PC content of chum salmon meat were 10.9±5.9% and 4.1±2.5%, respectively.

The molecular species composition of PCs isolated from chum salmon meat were obtained by HPLC/ESI-MS with a Gemini-5u-C18-110A (Table 5). The major components in the molecular species composition of PCs from chum salmon were DHA and EPA. However, in the saturated fatty acids, (SFA)/PUFA, and monounsaturated fatty acids, (MUFA)/PUFA, PC categories, 14:0, 16:0, 18:0, 16:1, and 18:1 species containing 22:6n-3 were more abundant than corresponding species containing 20:5n-3. These results are completely different from the sperm of Atlantic salmon and Chinook salmon in which 20:5n-3-containing molecular species were the major components (Bell et al., 1997).

Table 4. Lipid classes composition of oils extracted from chum salmon meat

	Whole body oil (%)	Meat oil (%)	Viscera oil (%)	The others oil (%)
Triglyceride	61.9 ± 18.6	50.3 ± 19.1	48.2 ± 15.0	79.1 ± 10.7
Cholesterol	2.2 ± 1.0	10.0 ± 0.1	4.1 ± 0.9	5.3 ± 2.2
Phosphatidylethanolamine	4.6 ± 1.9	10.9 ± 5.9	14.8 ± 3.7	6.2 ± 6.5
Phosphatidylcholine	5.9 ± 0.6	4.1 ± 2.5	20.2 ± 13.9	4.9 ± 3.6
The other phospholipids	24.5 ± 18.2	24.5 ± 16.8	12.0 ± 7.8	4.3 ± 3.4

Values are mean±SD (n=9).

Table 5. Molecular species composition of phosphatidylcholine isolated from chum salmon meat obtained by HPLC/ESI-MS with Gemini-5u-C18- 110A

RT (Time)	[M+Na]+	Molecular species	Peak area %
16.3	900.5	22:6/22:6	3.08
16.4	850.5	18:3/22:6	0.35
17.5	774.5	14:0/20:5	0.55
	800.5	16:1/20:5	0.36
18.3	826.5	18:2/20:5	0.28
19.1	800.5	14:0/22:6	1.53
19.3	826.5	16:1/22:6	1.30
22.1	814.5	15:0/22:6	-
22.5	700.5	14:0/14:0	0.26
23.4	802.5	16:0/20:5	14.08
24.1	828.5	18:1/20:5	-
25.5	828.5	16:0/22:6	26.54
26.3	854.6	18:1/22:6	-
28.8	804.5	16:0/20:4	1.42
	830.5	16:0/22:5	1.79
30.1	842.5	17:0/22:6	0.33
30.6	754.5	16:0/16:1	3.17
31.7	814.6	15:0/22:6	10.73
31.8	830.6	18:0/20:5	1.64
	780.6	16:0/18:2	0.84
34.6	882.5	20:1/22:6	0.66
35.1	856.6	18:0/22:6	2.63
35.8	768.6	15:0/18:1	0.57
39.8	858.6	18:0/22:5	-
42.3	782.6	16:0/18:1	23.69
44.7	808.5	18:0/18:2	-
59.4	810.6	18:0/18:1	4.19
76.1	770.5	17:0/16:0	-
Total (Each otherfatty acids/22:6)			44.07
Total (Each other fatty acids/20:5)			16.91

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