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Changes in the Fatty Acid Composition of Phospholipid in the Dried and Salted Mullet Roe during Processing and Storaging

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Abstract

Mullet roe was salted and dried by the conventional processing method. Mullet roe was first salted with soybean sauce containing 10% NaCl and then pressed down to be a 1.2cm of thickness. It was dried at 20° C under 3m/sec of aeration for 20 days. The lipids of the processed roe were fractionated by free and bound phospholipids. The contents of free and bound phospholipids were 9.30mg/100mg and 13.0mg/100mg respectively. The content of bound lipids were rapidly decreased than that of free fatty acids during processing and storaging. The major fatty acids of phospholipids were $C_{16:0}$, $C_{20:0}$, $C_{16:1}$, $C_{18:2}$ and $C_{20:5}$, whose contents were 6.64mg/100mg that occupied 72% of the total phospholipids. The ratio for the unsaturated fatty acids to the saturated ones of free phospholipids in fresh roe was 1.53 and it was decreased down to 0.34 in 9 weeks of storage. But the ratio of bound phospholipids was 1.04 of fresh roe and zero in 6 weeks. The content of essential fatty acids in bound phospholipids was 3.85mg/100mg occupying 75% of total essential fatty acids of the fresh roe, but they were totally destroyed during processing.

Key words : mullet roe, phospholipids, fatty acids

INTRODUCTION

Mugilidae is a coastal fish in the Indian, the Mediterean, the Pacific and the Atlantic oceans[1]. The grey mullet[2] (*Mugil japonicus*) is caught at the mouth of the Han, Chungchun and Youngsan rivers between late spring to early summer. The total amount of mullet caught in South Korea was 5,479 M/T in 1980 and 9,024 M/T in 1985[3], which shows that the amounts have gradually increased year after year.

The salted and dried mullet roe has been known to be one of the traditional unique foodstuffs in the area of Youngam-gun, Chonnam. The processing techniques for salting and drying were handed down from generation to generation. This product is not well known nationwidely because of lack of acknowlegement and special processing techniques in a family Gruger et al.[4] and Niemal and Hermans[5] reported that fatty acid of the roe product are composed of the odd-numbered fatty acids from $C_{13:0}$ to $C_{21:0}$. The proximal composition of mullet roe were 42% of crude protein and 40% of crude lipids of which major fatty acids were $C_{16:0}$ and $C_{18:1}$[6]. Joh and Ko[7] also reported that the major part of mullet roe oil was wax ester which occupied 20.5% of the total lipids.

De Konning and McMullan[8] reported that the fatty acid composition of phospholipid of rock lobster roe were very different from that of mullet roe.

The purpose of this study is to determine the fractional parts of the lipid contents and to analyze that changes the constitutional fatty acid composition of phospholipids during processing and storaging.

MATERIALS AND METHODS

Sample

Treatment of fresh roe

The grey mullets (*Mugil japonicus*, average length 76cm, weight 1.8kg) caught in May 1989 in the coastal area of Miam-myon, Youngam-gun, Chonnam.

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The fresh roe was picked from mullet ovary and weighed approximately 400 grams. This roe was soaked in the soybean sauce containing 10% salt for 20 hours. After washing with tap water, these were pressed down with one kg weighting plate for 1 minute for three times a day on the drying board for shaping.

Drying and storage

Sesame oil was painted twice a day on the surface of the shaped and salted roe with drying rack of cabinet drier[9]. The roe was dried by wind drying at 20° C, RH 70% and 3m/sec of aeration for 20 days. After drying, it was stored in a cold room (0±1° C) in order to retard the reaction of enzymes, fungi reproduction and lipid oxidation.

Chemicals

The standard chemicals of fatty acid methyl esters, silicic acid, Sephadex G-25, and the others were purchased from Sigma's and Shimadzu's.

Experimental methods

General components

The moisture content of roe was determined by the heat drying method, the crude protein content by Kjeldahl method and the ash content by electronic burning method[10].

Analysis of lipid

Total free and bound lipids

Free lipids were extracted from the mullet roe with diethyl ether for 12 hours at 60° C ; bound lipids were extracted from the residues of the free lipid extracted roe with a mixture solvent of chloroform-methanol-water (10 : 9 : 1, v/v/v) at 30° C for 12 hours. The contents of free and bound lipids were measured gravimetrically. The extracted lipids were stored in a captube at −1° C with N_2 gas.

Fractionation of lipids

Free and bound lipids were washed by the Folch method[11] and were fractionated into neutral, glyco- and phospholipids by the method of Roner and Nelson[12]. Ten grams of silicic acid (100mesh) was cleaned with water and then activated with methanol to remove the very small particles at 115° C for 12 hours and dried in a silica gel desiccator. The glass column (40cm × 2cm) was packed with the activated silicic acid and 100mg of lipid was applied. Neutral lipid, glycolipid and phospholipid were eluted with chloroform, acetone and methanol, respectively.

Finally, the fractionated layers of each lipid were washed again using Wuthier method[13]. The Wuthier' s washing solution made with chloroform-methanol-water (200 : 200 : 75, v/v/v) stopped separating the solution into two layers ; upper phase (UP) and lower phase (LP). 100mg of lipids were resolved with 5ml of LP solution and applied to the top of cilicic acid packed glass column (15cm (L) × 1cm (D)) and then washed with LP solution.

The lipid of LP solution was eluted with N_2 gas by 1 drop per second. The drained solution was dried under N_2 gas and the obtained lipid fraction was sealed with N_2 gas and then stored in a refrigerator at 0° C.

Analysis of fatty acids

The methyl esters of fatty acids were prepared by Metcalfe method[14] and the fatty acid composition were determined by gas liquid chromatography (Shimadzu GC-RLA). The 150mg of lipids was dissolved in the mixture of 4ml of 0.5N methanolic NaOH and 10mg of 10% BF_3-methanol solution for 5 minutes while heating, and then 10% NaCl solution were added. The upper layer of petroleum ether was evaporated under a vacuum evaporator. The obtained methyl esters were used for fatty acid analysis and the concentration were calculated by the comparative area to that of the standard ($C_{17:0}$).

Table 1. Operating conditions for gas chromatography

Instrument	Shimadzu GC-RIA
Detector	FID detector
Column size	2m (L) × 3mm (D)
Packing material	15% DEGS
Chart speed	10mm/minute
Column temperature	175~190° C (5° C/min., after 8.3min.)
Injection temperature	250° C
Detecting temperature	250° C
Carrier gas	N_2 gas, 20ml/min.

RESULTS AND DISCUSSION

Approximate composition of the salted mullet roe

The crude protein of fresh mullet roe was 23.9%. The crude lipid of the fresh mullet roe was 22.5%, which were a little more than 19.5% of dragon shark's roe and 21.05% of Considine's data[15]. The salted and dried mullet roe was 40% of crude lipid and 42.19% of crude protein, which was higher in protein and lipid than the other sea food products[16]. The higher lipid content of the product might be due to the addition oil during drying.

Lipid composition during processing and storaging

The separated free and bound lipids were fractionated as the neutral, glyco- and phospholipids. The free lipid of fresh mullet roe was 61mg/100mg, which is approximately doubled amount of the bound lipid, 35mg/100mg. The concentration of phospholipids in the free lipids were the least among the fractional lipids, which was in agreement with the result of No et al.[17]. The phospholipids of bound lipids were 12mg/100mg that is the similar result of No et al.[17].

Constitutional fatty acid changes of phospholipids

The constitutional fatty acid contents of phospholipids of free and bound lipids of the salted and dried mullet roe were determined during processing and storaging. The results are shown in Table 2 and Table 3.

The major fatty acids of free phospholipids were found to be $C_{16:0}$, $C_{20:0}$, $C_{18:2}$ and $C_{20:5}$, whose total amounts were 6.64mg/100mg, occupying 72% of the total fatty acids of fresh roe. Those of bound phospholipids were $C_{16:0}$, $C_{18:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$, whose total amounts were 12.40mg/100mg that occupied 91% of the total fatty acids of the fresh roe.

The analyzed unsaturated fatty acids of free phospholipids were only three kinds, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$, while that of bound phospholipids were $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{20:2}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$. The major polyenoic acid were $C_{18:2}$ and $C_{20:5}$ in both free and bound phospholipids.

Table 2. Changes in fatty acid composition of free phospholipids of mullet roe during processing and storaging

(mg/100)

Fatty acids	Weeks			
	0	3	6	9
8 : 0	0.11	0.11	0.05	0.02
10 : 0	0.12	0.29	0.27	0.21
12 : 0	0.27	0.38	0.46	0.19
13 : 0	0.05	0.04	0.03	0.03
14 : 0	0.11	0.10	0.59	0.22
15 : 0	0.25	0.40	0.18	0.29
16 : 0	1.37	1.35	1.43	1.59
17 : 0	0.03	0.13	0.10	0.27
18 : 0	0.03	0.05	0.02	0.05
19 : 0	0.02	0.02	0.03	0.41
20 : 0	1.19	1.02	1.08	0.99
21 : 0	0.08	0.16	0.07	0.06
Saturates	3.63	4.05	4.31	4.29
10 : 1	0.49	0.44	0.07	–
16 : 1	0.67	0.49	0.35	0.32
18 : 1	1.47	1.51	1.45	0.43
Monoenes	2.63	2.44	1.87	0.75
18 : 2	1.02	1.02	0.30	–
20 : 2	0.26	0.32	0.14	0.16
20 : 4	0.01	tr.	–	–
20 : 5	1.59	1.37	1.22	0.75
22 : 6	0.06	0.12	0.09	–
Polyenes	2.94	2.83	1.75	0.91

Table 3. Changes in fatty acid composition of bound phospholipids of mullet roe during processing and storaging

(mg/100mg)

Fatty acids	Weeks			
	0	3	6	9
8 : 0	0.07	0.02	0.03	0.01
10 : 0	0.14	0.33	0.28	0.40
12 : 0	0.19	0.10	0.08	0.20
14 : 0	0.10	0.15	0.28	0.20
15 : 0	0.31	0.58	0.47	0.28
16 : 0	1.75	1.96	1.90	1.38
17 : 0	0.02	0.31	0.42	0.44
18 : 0	1.93	2.18	2.40	1.73
19 : 0	0.23	0.36	0.28	0.26
20 : 0	0.40	0.40	0.55	0.19
21 : 0	0.13	–	–	–
Saturates	5.31	6.45	6.72	5.13
16 : 1	1.19	1.10	–	–
18 : 1	2.49	3.10	1.52	–
Monenes	3.68	4.20	1.52	–
18 : 2	3.85	1.10	–	–
Polyenes	3.85	1.10	–	–

Table 4. Comparison in free and bound fatty acid contents of phospholipids of mullet roe during processing and storaging

(mg/100mg)

Fatty acids			Weeks			
			0	3	6	9
Saturates		Free	3.63	4.05	4.31	4.29
		Bound	5.31	6.45	6.72	5.13
		Total	8.94	10.50	11.03	9.42
Unsaturates	Monoenes	Free	2.63	2.44	1.87	0.75
		Bound	3.68	4.20	1.52	–
		Total	6.31	6.64	3.39	0.75
	Polyenes	Free	2.94	2.83	1.75	0.91
		Bound	3.85	1.10	–	–
		Total	6.79	3.93	1.75	0.91
	Total		13.10	10.57	5.14	1.66
TUFA/TSFA		Free	1.53	1.41	0.82	0.34
		Bound	1.41	0.82	0.22	0.18
TPEA/TMEA		Free	1.12	1.15	0.96	1.21
		Bound	1.04	0.26	–	–
TEFA		Free	1.30	1.32	0.44	0.16
		Bound	3.85	1.10	–	–
		Total	5.15	2.42	0.44	0.61

TUFA/TSFA, total unsaturated fatty acid/total saturated fatty acids : TPEA/TMEA, total polyenoic acid/total monoenoic acids : TEFA, total essential fatty acids

The total fatty acid contents of phospholipids were 29.6% in alaska pollack, 45.8% in scumber, 17.5% in anchovy, 15.1% in sardine and 13.3% in mackerel[18]. But the total fatty acid contents of phospholipids were about 13.3% in this mullet roe, which was the same result in that of mackerel and the least than that of other fish.

The content of unsaturated fatty acids of phospholipids were 60% of that of total unsaturated lipid. Besides, $C_{18:2}$ and $C_{20:5}$ in free and bound phospholipids were rapidly destroyed during processing and storaging. The destroyed percent of unsaturated fatty acids in the phospholipids of the dried mullet roe was about 48.36 at 9 weeks. This destroyed result was very similar percent in the unsaturated fatty acids of tunna's phospholipids[19]. Odd numbered fatty acids of phospholipids in the mullet roe were $C_{13:0}$, $C_{15:0}$, $C_{17:0}$, $C_{19:0}$ and $C_{21:0}$[20]. Nessler and Sckulke[21], and Ramanuja and Herman[22] reported that the composition of mullet roe would have 10~20% of odd-numbered fatty acids in the total contents of fatty acids. This result was different with that of this study.

Table 4 shows the unsaturated fatty acid ratio to that of the total saturated fatty acids (TUFA/TSFA), polyenoic acid ratio to the total monoenoic acid (TPEA/TMEA). Total essential fatty acid changes during processing and storage of the processed mullet roe.

The ratio of the unsaturated fatty acids to that of total saturated fatty acids (TUFA/TSFA) of fresh mullet roe were changed 1.53 to 0.34 after 9 weeks in the free phospholipids and 1.41 to 0.18 after 9 weeks in the bound phospholipids. These results were similar with those of free phospholipids of snake head[23] but were different in the bound phospholipids.

The ratio of polyenoic acids to the total monoenoic acid (TPEA/TMEA) were constant all through the processing times in the free phospholipids and were decreased little by little during the processing times. But the content of polyenoic acids of phospholipids were totally destroyed after a processing periods of 3 weeks.

The mostly destroyed fatty acids was $C_{18:2}$ which had the most rapid destroying velocity in the total fatty acids. This was the reason why $C_{18:2}$ had the most rapid destroying velocity in the autooxidative reaction during drying times.

Total essential fatty acid contents of phospholipids was 5.15mg/100mg in the fresh roe, which was greater than that of glycolipids, 4.32mg/100mg.

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염건숭어알의 가공과 저장중 인지질의 지방산 함량 변화

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요 약

염건 숭어알을 10% 염농도의 간장에 침지하고 1.2cm 두께로 압착하여 20°C에서 3m/sec 풍속으로 음건하여 제조하였다. 제품의 지질을 중성지질, 당지질 및 인지질로 분획하고 유리인지질은 9.3mg/100mg, 결합인지질은 13.0mg/100mg함유하였으며 가공과 저장중에 빠른 속도로 감소하였다. 인지질을 구성하는 주요 지방산은 $C_{16:0}$, $C_{20:0}$, $C_{16:1}$, $C_{18:2}$와 $C_{20:5}$였으며, 이들 지방산의 함량은 6.64mg/100mg으로 총인지질 지방산의 72%를 점유했다. 불포화도는 유리 인지질에서 1.53이었고 결합 인지질에서는 0.34로 낮았다. 그러나 결합 인지질의 불포화도는 신선할 때는 1.04이었고 6주후에는 0에 가깝고, 필수지방산도 3.85mg/100mg으로 전체 필수지방산의 75% 정도이었으나 가공중에 거의 파괴되었다.