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Fatty Acid Changes of Glycolipids during Processing and in Storage of the Salted and Dried Mullet Roe

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Abstract

The salted and dried mullet roe was manufactured by the conventional processing method. The processing conditions were the salting with soybean sauce of 10% NaCl, 1.2cm of thickness, 3m/sec of air velocity, 70% of RH and 20℃ of wind-drying temperature for 20 days. The fractional compositions of free and bound lipids were classified in neutral, Glyco - and phospholipids of the processed roe. The fatty acid content of glycolipids was measured during processing and storage. Major fatty acids of glycolipids were $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ whose total amount was 7.71mg/100mg occupying 77% of the total fatty acids of glycolipids. The ratio of unsaturated fatty acid to the saturated fatty acid of bound glycolipids was 2.09 and that of free glycolipids was as low as about 0.92. The ratios of the polyenoic acids to the monoenoic acids were very low as 0.10-0.78. The essential fatty acids of bound glycolipids were 4.32mg/100mg and a very much decreased content of 1.46mg/100mg at 9 week storage time.

Key words : mullet roe, glycolipids, fatty acid.

INTRODUCTION

Mullet is a kind of commercial marine fish which is predominent all around the world[1], the coastal vicinity of the Indian, the Mediterenean, the Pacific and the Atlantic oceans. There are several kinds of species of grey mullet[2] (*Mugil japonicus*) in the coastal area of the Korean Pen-insula. They are caught in the mouth of he Han, Chungchun and Youngsan rivers in the season from late spring to early summer. The total amount of mullet fishing was 5,479 M/T in 1980 and 9,024 M/T[3] in 1985, which showed that the fish catching amounts gradually increased year after year.

Salted and dried mullet roe has been one of the traditional foodstuffs in the area of Youngam, Chonnam province, whose processing technique was handed down from generation to generation by special technicians with lack of sufficient knowledge and scientific background for consumers. Gruger et al[4]. and Niemal and Heman.[5] reported that the dried mullet roe had odd numbered fatty acids from $C_{15:0}$ to $C_{21:0}$. Baik[6] described the general composition as 42% of crude proteins and 40% of crude lipids which consisted of $C_{16:0}$, and $C_{18:1}$ as the major fatty acids.

Joh and Ko[7] also reported that the major part of mullet roe oil was wax ester as much as 20.5% of the total lipids.

De Knning and McMullan[8] insisted that the fat of rock lobster had very different composition from that of mullet roe.

They produced the peculiar dried mullet roe which has a long and semirounded thin shape in a Nakasaki area in Japan called Karasumi[9]. The salted and dried roe in the area of Youngam-gun is being processed traditionally by salting with 10% NaCl solution for 20 hours, washing with water, draining, forming a flat piece shape and spreading sesame oil on the surface of the salted roe perio-

dically during wind drying for 20 days.

There are several different conditions for making roe porducts, which affect the quality of the precessed roe. The purpose of this study is to determine the fractional lipid contents and to anlalyze the constitutional fatty acid contents of glycolipids during processing and storage times.

MATERIALS AND METHODS

Treatment of fresh roe

The grey mullet (*Mugil japonicus*, average length 76cm, weight 1.8kg) was caught in May 1989 in the coastal area of Miam-myon, Youngam-gun, Chonnam province. The fresh roe was 400g and was picked from mullet ovary. This roe was soaked in the soybean sauce of 10% salt for 20 hours. After washing with water, they were pressed for 1 minute 3 times a day with a drying board for shaping.

Drying and storage

Sesame oil was painted 2 times a day on the surface of the shaped and salted roe with the drying rack of a manufactured cabinet drier. The wind drying was carried out at 20℃, 3m/sec of air velocity, and 70% relative humidity for 20 days. After drying, it was stored in a cold room(0±1℃)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 others come from Sigma's and Shimadzu's.

Proximate analysis

Moisture was determined by heat drying method[10], crude protein by kjeldahl method[10], and ash content by electric burning method[10].

Extraction of total free and bound lipids

Total free lipids were extracted from the mullet roe with diethyl ether for 12 hours at 60℃. Bound lipids were extracted from the residues with a mixture solution of chloroform-methanol-water (10 : 9 : 1, v/v/v) at 30℃ for 12 hours.

The contents of free and bound lipids were measured gravimetrically. The lipids extracted were stored in a cap tube at -1 ℃ with N_2 gas.

Fractionation of lipids

Free and bound lipids were cleaned by Folch method[11] and were fractionated into neutral, glyco- and phospholipids by the method of Rouser et al[12]. Ten gram of silicic acid (100mesh) was cleaned with water, and heated with methanol to remove the unreasonable small particles at 115 ℃ for 12 hours, and finally dried in a silicagel desiccator.

After the glass column (40cm (L) ×2cm (φ)) was packed with silicic acid and 100 mg of lipids, the drained solution with chloroform was neutral lipids, that with acetone was glycolipids and that with methanol was phospholipids, in that order.

Finally, the fractionated layers of each lipid were washed once again by Wuthier method[13]. The Wuthier washing solution made of chloroform-methanol-water (200 : 100 : 75, v/v/v) stopped to separate it into two layers; upper phase (UP) and low phase (LP). 100mg of lipids was resolved with 5ml of LP solution and injected to the glass column (15cm(L) × 1cm(D)), and then washed with LP solution.

In washing, the lipid with LP solution was pressed with N_2 gas at the flow rate of one drop per second. The drained solution was evaporated with N_2 gas flow and the lipid was sealed with N_2 gas, and then stored in a refrigerator at 0℃.

Analysis of fatty acids

The fatty acids of fractionated lipids were determined by gas liquid chromatography (Shimadzu A-1), and the methyl esters of fatty acids were made by Metcalfe method[14].

150mg of lipids were resolved by heating with 4ml of 0.5N methanolic NaOH and 10mg of 10% BF_3-methanol solution for 5 minutes, and then added 10% NaCl solution.

Table 1. Operating conditions for gas chromatography

Instrument : Shimadzu A-1
Detector : FID
Column : 2m × 3mm I. D. 15% DEGS on chromosorb W, glass Column
Chart speed : 10mm/min
Coumn temperature : 175~190℃ (5℃/min, after 8.3 min.)
Injection temperature : 250℃
Detection temperature : 250℃
Carrier gas and flow rate : N_2, 20ml/min.

The upper layer of petroleum ether was taken with a syringe to be evaporated with a vacuum evaporator. The methyl esters obtained were used for gas chromatographic analysis (Table 1). The amounts of fatty acid methyl esters were measured by the comparative calculation to the standard ($C_{17:0}$).

RESULTS AND DISUCSSION

Approximate composition of salted mullet roe

The crude protein of fresh mullet roe was 23.9% and crude lipid 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 crude lipid of salted mullet roe was 40% and protein of the product was 42.19%, which were higher in protein and lipid than other sea food[16] products. The higher lipid content of the product ascribes to the added oil during drying.

Lipid composition during processing and in storage

The total lipid was separated into free and bound lipids. The free lipids of the fresh mullet roe was 61mg/100mg, which almost doubles the amounts of the bound ones (35mg/100mg). The extracted free and bound lipids were fractionated in neutral, glyco- and phospholipids.

In general, the amounts of neutral, glyco- and phospholipid are quite different depending upon species, size, and sectional parts of a fish body[17].

The fresh mullet roe had 11.9mg/100mg of glycolipids in free lipids, while those of bound lipids had 9.0mg/100mg, which had the same results of No et al[18].

The free and bound lipid contents increased during processing but the bound lipids rapidly decreased from 37.35mg/100mg to 22.59mg/mg in storage. In nine weeks, the free lipids sharply decreased as much as 15mg/100 mg. This was the major lipids decreased.

The reason for the lipid reduction during the processing and in storage is possibly that free lipids were present in the disassociated states and these were more susceptible to the lipid oxidation than the bound lipids.

The glycolipids of the bound lipids are not easily oxidized in comparison with phospholipids because the latter have polar dissolved phosphates which are more active to react with other unstable components in the oxidative reaction.

Constitutional fatty acid changes of glycolipids

Glycolipids of the salted and dried mullet roe had the fatty acids of free and bound lipids during processing and in storage, as shown in Table 2 and Table 3.

In fresh mullet roe, major acids of glycolipids in the free form were 1.03mg of $C_{18:0}$, 2.69mg of $C_{18:1}$, and 3.66mg of $C_{18:2}$ per 100mg oil. The sum of those acids was 7.78mg/100mg as 63% of total free fatty acids of glycolipids. The free fatty acids of the *glycolipids were less than those of neutral lipids*, particularly highly unsaturated fatty acids.

The amount of saturated fatty acid of the salted and dried mullet roe remained as all through the processing. But the unsaturated fatty acids were decreased extremely during processing and instorage. Especially, the polyenes had sharply decreased and had trace amounts in 9 week storage.

The major fatty acids of bound glycolipids were 1.03mg of $C_{18:2}$, 2.69mg of $C_{18:0}$ and 3.66mg of $C_{18:2}$

Table 2. Variations of fatty acid contents of free glycolipids of mullet roe during processing and in storage (mg/100mg)

Fatty acids	Weeks			
	0	3	6	9
8 : 0	0.08	0.06	0.06	0.06
10 : 0	0.03	0.03	0.03	0.04
12 : 0	0.05	0.03	0.03	0.04
13 : 0	0.11	0.01	0.00	0.01
14 : 0	0.18	0.17	0.16	0.17
15 : 0	0.32	0.36	0.33	0.34
16 : 0	2.54	3.19	3.27	2.73
17 : 0	0.17	0.18	0.09	0.12
18 : 0	1.31	1.75	1.22	1.51
19 : 0	0.11	0.09	tr.	0.05
20 : 0	1.38	1.28	1.10	1.15
21 : 0	tr.	–	–	–
Saturates	6.34	7.20	6.34	6.25
16 : 1	2.18	1.70	1.03	0.50
18 : 1	2.58	2.60	2.10	1.96
Monenes	4.77	4.30	3.13	2.47
18 : 2	0.65	0.66	0.30	–
20 : 3	0.39	–	–	–
Polyenes	1.04	0.66	0.30	–

per 100mg oil. The total amount of these acids was 7.32mg/100mg occupying 77% of the total bound fatty acids of glycolipids. The unsaturated fatty acids had also decreased rapidly during the processing in storage. Those unsaturated fatty acids of free and bound form in the glycolipids had shared about 77% of total fatty acids of glycolipids, which is typical in marine fish oil products. However, the cultured and natural eel had lower contents of unsaturated fatty acids of glycolipids[19], which was contrary to this study.

Besides, odd-numbered fatty acids were also found in the glycolipids such as $C_{13:0}$, $C_{15:0}$, $C_{17:0}$, $C_{19:0}$ and $C_{21:0}$, whose total amount was 0.7mg in free and bound glycolipids.

Unsaturated fatty acid rate (TUFA/TSFA), polyenoic acid rate (TPEA/TMEA) and total essential fatty acids (TEAA) of free and bound lipids were shown in Table 4.

Saturated fatty acids of glycolipids remained constant during processing periods. While unsaturated fatty acids gradually decreased during processing and storage, the polyenes of unsaturated fatty acids decreased sharply, Especially the unsaturated fatty acids of free glycolipids were profoundly destroyed in 9 weeks of storage.

The ratio of the unsaturated to the saturated fatty acids of bound form was 0.21~2.09, which were shown different results from those of eel which was 1.63~2.65[22] in the bound lipids. Monoenoic acids of free glycolipids were twice as much as those of bound form. But the amount of polyenoic acids of bound form was about 3~6 times higher than free acids, which is consistent with the

Table 3. Variations of fatty acid contents of bound glycolipids of mullet roe during processing and in storage (mg/100mg)

Fatty acids	Weeks			
	0	3	6	9
8 : 0	0.04	0.37	0.36	0.35
10 : 0	0.02	0.12	0.04	0.04
12 : 0	0.11	0.02	0.10	0.18
13 : 0	0.06	0.15	0.07	0.06
14 : 0	0.11	0.12	0.14	0.18
15 : 0	0.10	0.13	0.11	0.15
16 : 0	0.53	0.64	0.87	0.65
17 : 0	0.21	0.31	0.67	0.55
18 : 0	1.03	1.09	1.31	1.25
19 : 0	0.18	0.19	0.01	–
20 : 0	1.52	0.78	0.76	0.73
21 : 0	0.15	0.94	–	0.84
Saturates	3.06	4.86	4.08	5.03
16 : 1	0.03	–	–	–
18 : 1	2.69	2.30	2.05	0.59
Monenes	2.72	2.30	2.05	0.59
18 : 2	3.66	2.32	1.75	0.46
20 : 2	0.02	–	–	–
Polyenes	3.68	2.32	1.75	0.46

Table 4. Comparison in free and bound fatty acid contents of glycolipids of mullet roe during processing and in storage (mg/100mg)

Fatty acids			Weeks			
			0	3	6	9
Satutrates		Free	6.34	7.20	6.34	6.25
		Bound	3.06	4.86	4.68	5.08
		Total	9.40	11.86	11.02	11.33
Unsaturates	Monoenes	Free	4.77	4.30	3.13	2.47
		Bound	2.72	2.30	2.05	0.59
		Total	7.49	6.60	5.18	3.07
	Polyenes	Free	1.04	0.66	0.30	–
		Bound	3.68	2.32	1.75	0.46
	Total	Total	4.72	2.98	2.05	0.46
			12.21	9.58	7.23	3.53
TUFA/TSFA		Free	0.92	0.69	0.38	–
		Bound	2.09	0.95	0.75	0.21
TPEA/TMEA		Free	0.22	0.16	0.10	–
		Bound	0.08	0.11	0.36	0.78
TEFA		Free	0.66	0.66	0.30	–
		Bound	3.66	2.32	1.75	0.46
		Total	4.32	2.98	2.05	0.46

result of eel's[20] coincide.

The polyenoic acid content of glycolipids showed different results from those of sardine, which had 36.9~45.9%[21] of total lipids.

The essential fatty acids of bound glycolipids had 3.66mg/100mg in fresh roe and decreased to 0.46mg/100mg after 9 week storage times. The major fatty acid of bound glycolipids was $C_{18:2}$ which agreed with that in bound lipids of eel[20]. But the essential fatty acids of free glycolipids was small.

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염건숭어알의 가공과 저장중 당지질의 지방산 함량변화

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요 약

염건 숭어알은 전통적인 방법으로 제조하였다. 제조 조건들은 10%염농도의 간장에 염지하고 두께 1.2cm 정도로 압착하여 70% 습도에서 3m/sec의 풍속 및 20℃에서 음건하였다. 가공과 저장기간의 유리 및 결합지질을 분리하여 다시 중성, 당 및 인지질로 분획하였다. 주요구성 지방산은 $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, 과 $C_{18:2}$이며 합계량이 7.71mg/100mg 으로 당지질의 77% 정도 점유하였다. 불포화도는 결합당지질은 2.09로 유리당지질의 0.92와 차이가 많았다. 다가불포화 지방산비는 0.10-0.78로 낮았으며 필수지방산함량은 결합당지질의 신선어란 4.23mg/100mg에서 저장 9주째는 거의 소멸되었다.