第4卷第3期 中国水产科学 Vol.4, No.3
1997年9月 JOURNAL OF FISHERY SCIENCES OF CHINA Sep., 1997

THE EFFECT OF FISH QUALITY ON THE COMPOSITION AND OXIDIZATION OF FISH OIL

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ABSTRACT The effect of fish quality on the composition and oxidization of fish oil was studied by using fish oil made from different quality of fish. The oxidization level i.e. chemiluminescence (CL) – intensity of fish oil increased as fish was of bad quality. No significant correlation was observed between EPA, DHA content and CL – intensity. The color of each kind of fish oil was from light to dark according to the deteriorated degree of fish. The content of dienes, free fatty acids and diglycerides increased when the fish was bad. The triglyceride content of the oil was negatively correlated to the total voloatile N of fish. The oils produced from deteriorated fish were easily oxidized.

KEYWORDS Fish quality, Fish oil, Composition, Oxidization, Chemiluminescence (CL)

As far back as 1956, some data were published showing the intake of oil had cholesterol-lowering effect for humans. This was the first indication that fish oil and diet were some help against coronary heart diseases[1]. However, it was not until 1972, when Dyerberg published his findings that the reason for the low incidence of coronary heart disease in Greenland was related to their diet, especially to their high intake of ω – 3 polyunsaturated fatty acid eicosapentaeonic acid (EPA), docosahexaenoic acid (DHA)', only then did the scientific community become aware of the health – related roles of the ω – 3 fatty acids. This caused an interest in high quality fish oil.

Today fish oil is a by – product from the fish meal industry. When the raw material arrives at the factory, it is usually several days old and also may take one or two days until it is processed. It is obvious that lipid deterioration occurs through degradative changes of fish[2,3], so the quality of crude oil correlates with the quality of fish[4]. The previous study showed that the content of free fatty acid increased and the color darkened with the degradative fish. However, the lipid – classes composition and oxidization of fish oil made from different quality of fish has not been investigated.

收稿日期:1995 – 12 – 14。

The aim of this experiment was to examine how the fish quality affeted the chemical composition and oxidization of fish oil.

1 Material and Methods

1.1 Material

The fresh herring was collected at a herring oil factory in Bergen, Norway. We stored a batch of herring in insulated containers (0.5m^3) below 20℃ throughout a period of 3 weeks and we performed analysis on the raw fish every week. At the time of analysis, i.e., at time zero (the fresh fish, oil sample 1$^{\#}$) and at week 1 (oil sample 3$^{\#}$), 2 (oil sample 4$^{\#}$) and 3 (oil sample 5$^{\#}$), fish oil samples were produced at the pilot plant at SSF. In the meantime we took fish oil produced at other factory (oil sample 2$^{\#}$) tobe a contral sample.

1.2 Analysis methods

1.2.1 The chemiluminescence (CL) intensity of oil samples was measured using a luminometer (LKB1251, Wallacoy, Turku, Finland). Connected to a dispenser (LKB1291, Wallacoy, Turku, Finland). We used 50mg fish oil with 800ul tertiary butyl alconho (TBA) soloution and three drops of hypochlorite sodium solution (10%)[5].

1.2.2 The composition of fatty acids was measured by using standard method with gas liquid chromatography (GLC). The capillary columns used were at least 25m long and coated with aluminum foil. The carrier gas was helium. The retention time and area of each peak were measured by an integrator. Individual fatty acid was identified and quantified by a computer. The analysis was performed with internal standard (IS=23:0).

1.2.3 The composition of the lipid – classes of FO i.e. triglyceides (TG), diglycerides (DG), monoglycerides(MG), free fatty acids (FFA) and phospholipids (PL) was anaylzed with TLC – FID (chromarods, Iatrtoscan TH – ID). Neutral lipid mix and phospholipid mix standard samples were purchased from supelco (Bllefoutr. PA. USA). The following solvent systems were used: diethyo – ether – petroleum – ether – formic acid (35:75:1). After 1 (ul) of sample solution was spotted by means of micro – syrine, the chromarods were developed in a development tank at room temperature with solvent system (15min), then dried at room temperature[6].

1.2.4 Lag – time was determined with Rancimat (Rancimat 617) (80℃ for 20 hours. air – pressure 16L/H).

1.2.5 The oil – samples (15ml) were incubated at room – temperature in sealed Erlen – Meyer flasks (1.5 liter flasks for 90 hours) and the consumption of O_2 and the total pressure reduction inside the flasks were logged every second hours (two parallels per oil – samples).

1.2.6 Total Volatile N, Acid Value (AV) and Peroxide Value (PV) were analysed with AOCS method.

1.2.7 Conjugated bonds (Dienes, Triens) ($E^{1\%}$1cm): The contents of conjugated dienes and triens were measured respectively with the U.V absorption at 232nm and 269nm.

2 Results

Table 1 shows some analytical data on the fish – material and the respective oil samples.

Samples 1# and 2# had the same fresh herring but were processed at different plant. The total volatile N was increased with the time of storage. The content of comjugated trienes and in particular the content of dienes increased when the fish was of bad quality. Moreover, the color of the oil, were significantly influenced by the fish quality, i.e. the color of oil 1# and 2# was light yellow and that of 3# and 4# was brownish and that of 5# was dark brown. Fig.1 shows the absorption of different oil at different wave length. The darker the color of oil, the wider the absorption of wave length.

Table 1 The characteristic of fish and the respective oil

Sample#	1	2	3	4	5
Storage ume of the fish (weeks)	0	0	1	2	3
Total volatile N of fish before proces (mg/100g)	11	11	63	99	153
AV of fish oil	7	5	7	15	- - *
PV of fish oil (m.mol. O/kg)	3.4	2.8	2.2	1.0	1.2
Conjugated bonds: Dienes	6.77	6.63	7.76	11.05	17.82
Conjugated bonds: Trienes	0.21	0.21	0.27	0.61	0.85

* Was not analyzed due to the color intensity of fish oil

The analyses of the fatty acids are shown in table 2. Comparing the contents of EPA and DHA with each other among all the samples, we didn't find they were different significantly ($P>0.05$). And there were no significant differences among the total saturated, monounsaturated, PUF (n-6) and PUFA (n-3). It was shown that the composition of fatty acids was less influenced by the fish quality.

The results of the CL-analysis are shown in table 3. The CL-intensity or the oxidization level. increased significantly ($P<0.05$) in the oil with the deterioration of the raw fish. In order to make comparison easlly, we analyzed the CL-intensity after incubation. The CL-intensity increased significantly after the Rancimat incubation.

The composition of lipid-classes of the fish oil(%), i.e., triglycerides (TG), diglycerides (DG), monolycerides(MG), free fatty acids (FFA) and phospholipids (PL), was analyzed and the results are shown in table 4. The FFA and DG-content increased sinificantly and the TG-content of the oil was negatively correlated to total volatile N of fish.

The oil-samples were incubated in Rancimat. The fatty acid amalyses of the oil-samples after incubation were performed with is and the results are shown in table 2. The lag-time during the Rancimat-incubation, i.e., the assumed oxidization-resistance of the respective oil-samples without any antioxidant supplementation is shown in table 5. It was obvious that the oil produced from deteriorated fish was oxidized more easliy. The content of polyunsaturated fatty acies decreased significantly after the Rancimat incubation, but the oil-samples produced from deteriorated fish seemed to be less influenced by this treatment.

The oil-samples were incubated at room-temperature in sealed Erlen-Meyer flasks, and the results are shown in table 6. The oxygen and pressure in the flasks of oil produced from deteriorated fish decreased quickly. The oil containing more free fatty acids was easily oxidized.

Table 2　The composition of fatty acids of oil－samples before and after incubation

Oil samples#	1		2		3		4		5	
	Before	After	Before	After	Before	After	Before	After	Before	After
8:0										
10:0										
12:0	0.1		0.1		0.1		0.2		0.1	
14:0	8.5	8.2	7.3	9.8	8.1	9.8	9.1	7.8	9.3	9.8
16:0	11.9	9.4	11.1	12.2	11.9	12.4	12.5	11.5	12.0	14.0
18:0	0.8	0.5	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.9
20:0+18:3n－6	0.2	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1
16:1n－7	4.1	2.6	3.9	3.6	4.1	3.6	4.2	3.6	4.0	4.6
18:1(n－9)+(n－7)+(n－5)	11.9	7.5	11.5	10.1	11.9	10.0	12.3	10.1	12.2	12.7
20:1(n－9)+(n－7)	14.3	8.1	13.8	10.6	13.6	12.0	15.0	12.2	14.1	14.1
22:1(n－11)+(n－9)+(n－7)	21.5	12.7	20.7	16.6	21.5	18.1	22.4	18.7	21.1	21 2
24:1(n－9)	1.1	0.4	1.1	0.4	0.8	0.4	1.0	0.3	1.2	0.1
16:2n－4	0.2	0.0	0.2	0.0	0.2	0.0	0.3	0 0	0.2	0.1
16:3n－4	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
16:4n－1	0.3	0.3	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.1
18:2n－6	1.0	0.2	1.0	0.4	1.1	0.3	1.0	0.4	1.0	0.7
18:3n－6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:2n－6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1
20:3n－6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n－6	0.1	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.5	0.0
22:4n－6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n－3	0.9	0.0	0.9	0.3	0.3	0.1	0.9	0.2	0.8	0.4
18:4n－3	2.4	0.3	1.8	0.3	2.1	0.4	2.2	0.3	2.0	0.8
20:3n－3	0.1	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.1	0.4
20:4n－3	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.8
20:5n－3	5.2	0.0	5.2	0.0	5.3	0.0	5.5	0.0	4.8	1.8
21:5n－3	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.1	0.2	0.0
22:5n－3	0.5	0.0	0.0	0.0	0.4	0.0	0.5	0.0	0.4	0.0
22:6n－3	5.2	0.1	5.3	0.1	5.2	0.1	5.3	0.5	4.9	1.1
Sum saturnated	21.4	18.1	19.5	22.8	21.1	23.0	22.8	20.2	22.4	24.8
Sum monosaturnated	52.8	30.8	50.9	41.3	51.8	43.9	54.8	44.9	52.5	52.7
Sum PUFA (n－6)	1.3	0.3	1.3	0.6	1.3	0.5	1.3	0.5	1.7	0.8
Sum PUFA (n－3)	14.9	0.8	14.2	1.0	13.9	1.1	15.0	1.9	13.6	4.5
Sum PUFA－tot.	16.8	1.1	16.1	1.6	15.7	1.6	16.9	2.4	15.8	5.5
Total sum	91.0	50.0	86.5	65.6	88.6	68.5	94.5	667.4	90.7	82.9

Table 3　The comparision of CL－intensity before and after incubation

Oil sample#	CL－intensity	
	Before incubation	After incubation
1	927 ± 38	1978 ± 120
2	1895 ± 85	1926 ± 23
3	1127 ± 40	1806 ± 106
4	2430 ± 38	3989 ± 93
5	4253 ± 102	7137 ± 162

Table 4 The composition of the lipid－classes of oil samples

Oil sample#	1	2	3	4	5
TG	97.5±0.2	95.8±0.6	90.9±1.8	84.4±0.0	61.1±11.0
DG	0.0±0.0	1.0±1.4	2.1±1.2	5.0±2.4	9.8±2.3
MG	n.d.*	n.d.	n.d.	n.d.	n.d.
FFA	0.9±0.4	2.7±0.2	4.4±0.8	9.4±0.7	21.3±0.9
PL	n.d.	n.d.	n.d.	0.2±0.2	5.1±3.6

* not detected

Table 5 The lag－time of oil－samples

Oil sample#	1	2	3	4	5
Mean value for lag－time (hours at 80℃)	8.7±0.3	8.8±0.7	7.4±0.6	1.9±0.2	1.0±0.1

Table 6 Results of The respective duplicates (a/b)

Oil sample#	1	2	3	4	5
O_2－reduction(%)	6.2	5.2	5.5	17.0	24.4
Pressure reduction(mbar)	10	12	13	41	57

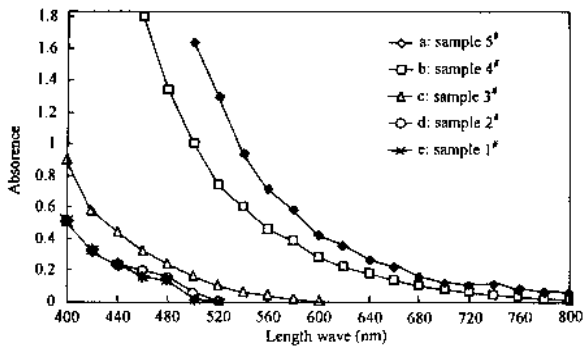

Fig. 1 Absorption of different oil at different wave length

3 Discussion

The color of fish oil mainly comes from the following two fatcors, one from its own and the other from its chemical change. We can see that oil 1# and 2# had light－color, but oil 3#, 4# and 5# showed brownish or dark－brown－color. This meant that fish oil 3#, 4# and 5# contained not only natural color but also color caused by chemical change in degrading fish and by chemical interaction between lipid oxidation products and secondary/or primary amino－groups of proteins/amino acids[7]. Fig. 1 showed that for oil 1#, 2# the absorption of wave length was from 400－520nm, but for oil 4# and 5# the absorption of wave length was from 480－800nm. This was caused mainly by some chemical substances in the oil when the freshness of fish was decreased. Reece. P. found that during the storage time and deterioration of the raw fish haemin was liberated from haemoglobin of the blood cells. The haemin molecule was soluble in lipids and the oil became brownish[8].

In our experiment, oil 1#, 3#, 4#, and 5# were processed at SSF, but oil 2# was processed at other factory. Previous study[4] had shown that the quality of crude fish oil was dependent on storage and handling of the fish prior to processing, the types of fish processing plant and the efficiency with which it was operated and the storage of handiling of the crude

oil. It was also demonstrated in our experiment.

It is easy to see that the crude fish oil contains some amount of free fatty acid that can have deleterious effect on the final product. Free fatty acid is the product of hydrolysis. It is oxidized more easily than conjugated fatty acid. The amount of the free fatty acids varies mainly in acordance with the freshness of the raw material used in the fish oil production. Moreover, the content of triglyceride in fish oil is negatively related with that of free fatty acid, because free fatty acid is the product of hydrolysis of triglycerides.

The method of incubation provides a rapid way to determine oxidized stability of defferent fish oil. Also the higher quality of fish oil with light－color and bland flavor has good oxidized stability. And for refiner, high quality fish oil can minimize production costs, i.e., low chemical usage and a short production. From table 6, it can be seen that the oxygen of flask containing oil $4^{\#}$, $5^{\#}$ decreased significantly ($P<0.05$). This meatnt that oil $4^{\#}$ and $5^{\#}$ could be easily oxidized. The results are consistent with high amount of free fatty acids.

The method that hypochlorite sodium induces and luminol (5－amino－1.2.3.4－tetrahydrophtalazin－1.4－dion) sensitizes CL of fish oil is a sensitive one to evaluate rancidity. The analytical method is simple and rapid to perform[5]. The results of CL－analysis are consistent with the total volatile N of the fish. We made the comparison of CL－analysis before and after incubation. The value of CL－intensity increased significantly with the oxidized－process. Oil $4^{\#}$ and $5^{\#}$ showed intense CL. It was confirmed that oxidizative deterioration contributed to CL observed in fish oil, since the CL－intensity increased in all fish oil in accordance with incubation.

On the other hand, all fish oil contained almost the same content of EPA and DHA that was susceptible to oxidization. And no signifficant correlation was observed between EPA, DHA content and CL－intensity, because all fish oil had significant CL－intensity. Usuki. R. once reported that the oxidative deterioration of edible oil and fried foods might be determined by measuring CL－intensity[9]. Moreover, metal, natural antioxidants, etc., might affect CL in addition to oxidative deterioration in oil.

It is considerable that the fish oil $5^{\#}$ contains high free fatty acid and its CL－intensity is the highest among the five samples. However, after Rancimat, the content of EPA and DHA is 1.8% and 1.1% respectively. The decrease of EPA and DHA in sample $5^{\#}$ is the smallest. This is obscure and needs further study.

Based on the above results, it may be concluded that the composition and oxidization of fish oil are influenced by the fish quality. The CL－intensity increases significantly in the oil with the deterioration of raw fish. However, the AV, PV and the fatty acid composition of the oil are less influenced. The content of dienes, FFA and DG increases when the fish is of bad guality, and the TG content of the oil is negatively correlated to the total volatile N of the fish. Also, light－colored fish oil can be obtained when the fish is of high quality. The oil produced from deteriorated fish is easily oxidized.

ACKNOWLEDGMENTS

The authors thank Mr. Atle Nilsen at Norwegian Herring Oil and Meal Industry Research Institute for his help at this research.

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鱼体质量对鱼油组成及氧化特性的影响

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摘 要 本文以不同质量的鱼及生产出的鱼油为原料，研究了鱼体质量对鱼油组成及氧化特性的影响。氧化程度即鱼油的化学发光强度随鱼体质量下降而上升。化学发光强度与EPA、DHA含量无明显关系。随鱼体质量下降，鱼油颜色由浅而深，且共轭二烯系含量、游离脂肪酸和甘油二酯含量增加。鱼油中甘油三酯与鱼体总挥发性氨基氮呈负相关。由低质鱼生产的鱼油较易氧化。

关键词 鱼体质量，鱼油组成，氧化特性，化学发光强度