KOREAN J. FOOD SCI. TECHNOL.
Vol. 19, No. 5 (1987)

Separation of EPA and DHA from Fish Oil by Solubility Differences of Fatty Acid Salts in Ethanol

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Abstract

Fatty acid fraction rich in ω-3 polyunsaturated fatty acids (ω-3, PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) could be obtained by saponification of fish oil in ethanol containing alkali hydroxide followed by cooling and filtration of the resultant solution. Fatty acid compositions of fish oil and the concentrates suggest that the ratio of number of double bonds to carbon number in a fatty acid molecule is a more important factor than the degree of unsaturation or the chain length in determining the solubility of fatty acid salts in ethanol. Water content in ethanol affected significantly the efficiency of the separation with respect to yield and content of EPA and DHA in the concentrates; the lower the water content, the higher the efficiency. It was, however, influenced little by cooling procedure and temperature which the saponified solution experienced during the crystallization. Under an optimal condition, the contents of EPA and DHA in the concentrate increased by 2.4 and 2.6 times, respectively, as compared with those in sardine oil.

Introduction

Epideniological studies have revealed that the Greenland Eskimos and Japanese fishermen who were consuming 200-400*g* fish/day were less prone to the coronary heart disease as compared with the Denmark Eskimos and Japanese farmers who were consuming limited amount of fish.[1-4] Later study indicated that this effect was resulted from the high content of ω-3 polyunsaturated fatty acids (ω-3, PUFA) contained in fish oil, especially eicosapentaenoic acid (EPA, $C_{20:5}$) and docosahesaenoic acid (DHA, $C_{22:6}$).[5] One serious problem in using the two acid components as a cardioprotective agent is that only high-level administration is effective for the prevention of cardiovascular disease, which stimulated many separation studies.

Separation methods of fatty acids on an industrial scale include panning and pressing, fractional distrillation, low temperature solvent crystallization, water emulsification separation, urea separation and metallic-salt solvent separation. Among them, the last two methods based on urea adduct formation and solubility differences of fatty acid salts in solvent have been developed and applied for the concentration of EPA and DHA from fish oil.[6] In commercial production, sodium-salt solvent separation is the overwhelmingly preferred choice for the simultaneous concentration of the two components because the procedure is simple and requires minimal equipment.[7]

This study was performed to reveal the separation principle of the sodium salt method[7] and to investigate the effect of water content in alcoholic alkali hydroxide solution and cooling procedure and temperature on the yield and the contents of EPA and DHA in the concentrate.

Materials and Methods

Saponification and Extraction

Saponification of oil and extraction of fatty acid salts were carried out according to the procedure of a Japanese patent[7]. Four *g* of sodium hydroxide were dissolved in 140*ml* of ethanol containing 0.2-15% (*v/v*) water. To this solution, was added 20*g* of sardine oil (Partially refined sardine oil, which is easily available and is rich in the desired EPA and DHA[8], was a kind gift from Iwha Oil & Fat Ind. Co., Ltd.) and the mixture was refluxed for 2 hr at 82°C. With the cooling of the saponified solution, it was solidified. The solid fraction was filtered off through a glass filter and thoroughly washed with ethanol. The combined filtrate enriched in particular fatty acids was than evaporated in a vacuum rotary evaporator at 35°C.

Recovery of ω-3 PUFA

Fifty *ml* of water and 20 *ml* of hexane were added to the dried soaps and after stirring for 10 *min*, the upper layer containing unsaponifiable matter was removed. Then pH of the lower layer was adjusted to 2.0 with the dropwise addition of hydrochloride solution. By addition of hexane, the free fatty acids formed were extracted and the hexane layer was separated. This procedure was repeated. The extracts were combined, washed with water, and concentrated by evaporating the solvent.

Analysis of fatty acid composition

According to the procedure of AOCS[9], fatty acids were esterified and the methyl esters were analyzed with a Varian gas chromatograph, model VISTA 6000. A stainless-steel column (3*m* x 2*mm* id) packed with silar 7 CP on Chromosorb W-HP (Supelco Inc., Bellefonte, PA, USA) was operated by temperature programming (1.5°C/*min*) from 185 to 235°C. The response of flame ionization detector was analyzed with a Data System, model DS601 of Varian and each component was identified by comparing the retention times of the standard methyl esters of fatty acids (Supelco Inc.).

Results and Discussion

Various methods of the metallic salt separations have been introduced in isolating saturated and unsaturated fatty acids. In the past, the application of the procedures on a large scale was mainly aimed at the removal of the undesirable PUFA which is vulnerable to oxidation. A Japanese patent[7], however, introduced one of the modified methods applicable to the concentration EPA and DHA from fish oil. Table 1 shows the fatty acid compositions of sardine oil and PUFA concentrate prepared according to the procedure of the patent in which the use of absolute ethanol instead of ethanol containing 5% (*v/v*) water was the only difference. In sardine oil, palmitic acid was the major component with EPA being the next most abundant, while EPA and DHA were the most and secondly abundant components, respectively, in the concentrate. About 76.0% of the fatty acids in the concentrate was ω-3 fatty acids consisted of EPA, DHA, and other *w*-3 PUFA such as $C_{16:4}$, $C_{18:3}$, $C_{18:4}$, $C_{20:4}$, and $C_{22:5}$.[10]

Separation of fatty acids is generally based on two factors, carbon chain length and degree of unsaturation. An examination of the difference in fatty acid compositions of sardine oil and the concentrate reveals that degree of unsaturation (i.e., number of double bonds) rather than chain length is a more important factor for the concentration of EPA and DHA by sodium salt method (Table 1). However, the principle of separation of this procedure can be more satisfactorily explained by introducing a concept of the ratio of number of double donds to carbon number. If we define the concentration factor as the ratio of percentage of a fatty acid in PUFA concentrate to that

Table 1. Fatty acid composition of sardine oil and PUFA concentrate obtained by sodium salt method (area %)

Fatty acid*	Sardine oil	PUFA concentrate**
14:0	6.1	1.6
15:0	0.7	0.3
16:0	18.4	1.6
17:0	1.4	0.5
18:0	4.0	1.3
14:1	0.1	0.1
16:1	7.7	5.0
17:1	1.2	0.7
18:1	13.5	5.0
20:1	4.3	1.4
22:1	2.0	0.6
18:2w6	1.9	1.2
20:2w6	0.3	0.2
18:3w3	1.0	1.1
18:3w6	0.5	0.4
20:3w6	0.1	0.1
16:4w3	1.2	2.0
18:4w3	3.3	6.7
20:4w3	0.9	1.4
20:4w6	1.7	1.6
22:2w6	1.4	1.3
20:5w3	14.2	34.1
22:5w3	1.9	2.9
22:5w6	0.4	0.7
22:6w3	10.7	27.8
unknown	1.1	0.4
unsaturated	68.4	94.7

* Number of carbons: number of double bonds.
** The fraction was obtained by saponifying sardine oil in absolute ethanol containing sodium hydroxide followed by rapid cooling to 10°C.

in sardine oil, it was highly correlated (r = 0.949) with the aforementioned ratio as shown in Fig. 1, in which the concentration factors of all the cases in Table 3 were calculated on the basis of the fatty acid compositions of each concentrate and sardine oil (the fatty acid compositions of ω-3 PUFA are not shown). It can be thus inferred from the result that sodium soap of a fatty acid is more soluble in ethanol at ambient temperature as the number of bouble bonds in a fatty acid molecule increases, and as the chain length decrease if the degree of unsaturation is the same.

It was argued to be essential that at least ca.4-5% water be present to effect rapid and complete saponification of fats and oils with alcohols.[6] By increasing water content, however, the efficiency of the sodium salt method may be lowered because all the soaps of acids are soluble in water. These two facts imply the existence of an optimal water content for the method. When PUFA concentrates were prepared by saponifying oils with ethanol containing 0.2, 1.5, 5.0, 10.0, and 15.0% of water respectively, both the yield and the contents of EPA and DHA in the concentrate were high in drcreasing order of water content (Table 2). For example, when absolute ethanol which inherently contains ca. 0.2% water was used, the contents of EPA and DHA increased by 2.4 and 2.6 times, respectively, with the yield of 84.5% as related to the sum of the two acids.

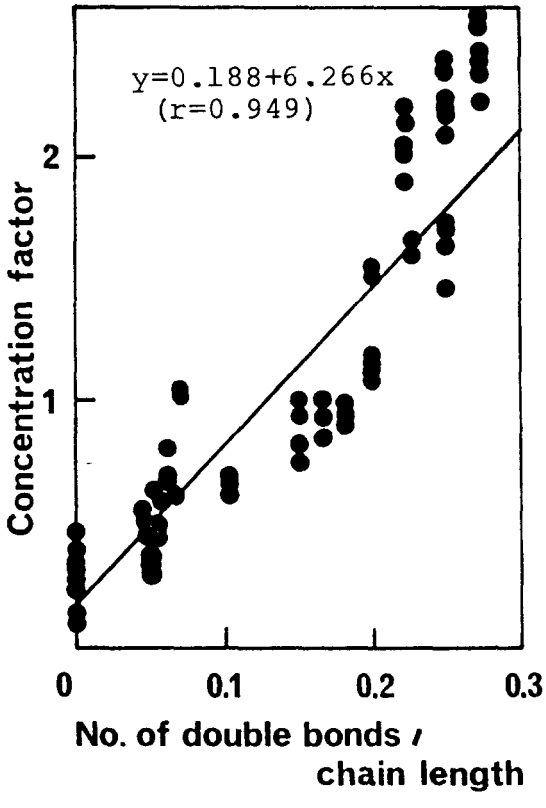

Fig. 1. Correlation between the ratio of number of double bonds to carbon number in a fatty acid molecule and the concentration factor of fatty acids from sardine oil concentruction factor is defined as the ratio of percentage of a fatty acid in ω-3 PUFA concentrate to that in sardine oil

The basic principle of separation of EPA and DHA by this method is that alkali salts of less saturated acids crystallize more readily than do those of polyunsaturated acids containing four or more double bonds when the saponified solution is cooled. Thus the effect of cooling temperature and procedure on the content of ω-3 PUFA in the concentrate and the yield were also investigated. The acid compositions of PUFA concentrate which experienced different treatments are shown in Table 3. As is apparent from the result, neither cooling procedure nor cooling temperature in the range of 25 to −15°C influenced significantly the contents of EPA, DHA, and other ω-3 PUFA and the yield, suggesting that the ambient temperature would be a practical choice. Table 3 also shows that two-step cooling, i.e.a saponified solution was firstly cooled to 10°C and the filtrate of it to −15°C did not improve the procedure in terms of the contents of EPA and DHA in the concentrate.

This sodium salt solubility method is of value for the commercial production for EPA and DHA enriched fraction from fish oil because the procedure is simple and needs minimal equipment. The ω-3 PUFA fraction obtained in this study could be a very useful as a cardioprotective foodstuff and also for other medical aplications.[11] On the other hand, a considerable number of investgations of the solubilities of individual fatty acid salt at various temperatures in a wide variety of solvents will give a more sound clue as to describe the separation principle and to improve the efficiency of the method.

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Table 2. Effect of water content in ethanolic sodium hydroxide solution on the enrichment of w-3 PUFA

Water content in ethanol(%)	Fatty acid composition (area %)			Concentrate obtained(g)*
	EPA	DHA	Other w-3 PUFA	
Sardine oil	14.2	10.7	8.3	
0.2	34.1	27.8	14.0	6.8
1.5	33.0	25.9	13.9	6.7
5.0	31.1	24.2	14.3	6.8
10.0	25.7	19.4	12.5	6.4
15.0	22.5	17.6	11.5	6.1

* Concentrate obtained from 20g of sardine oil.

Table 3. Effect of cooling temperature and procedure on the enrichment of w-3 PUFA

Cooling temperature (°C)	Cooling rate (°C/min)	Fatty acid composition (area %)			Concentrate
		EPA	DHA	Other w-3 PUFA	
25	1	32.5	25.6	14.3	7.6
10	0.5	33.6	27.1	14.5	7.1
10	2	34.1	27.8	14.0	6.8
0	0.5	33.7	27.3	14.6	7.0
0	2	34.2	28.0	14.1	6.6
−15	2	34.6	28.3	14.6	6.6
−15*	2	35.7	28.8	14.9	6.2

* The saponified solution was firstly cooled to 10°C and the filtrate of it was cooled further to −15°C.
** Concentrate obtained from 20g of sardine oil.

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(Received Sept. 3, 1987)

에탄올에 대한 지방산염의 용해도 차를 이용한 EPA와 DHA의 농축방법

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알칼리 수산화물이 용해되어 있는 에탄올을 사용하여 어유를 비누화시키고 이를 냉각·여과함으로써 ω-3고도불포화지방산 특히, EPA와 DHA가 농축된 지방산 농축물을 얻을 수 있었다. 정어리유와 농축물의 지방산 비교·분석한 결과 지방산 분자의 이중결합 수 또는 탄소사슬 길이보다는 탄소사슬 길이에 대한 이중결합 수의

비율이 에탄올에 대한 지방산 나트륨염의 용해도를 결정짓는 보다 중요한 인자라는 점을 알수 있었다. 비누화 반응의 용매인 에탄올 내의 물함량은 분리효율에 커다란 영향을 끼쳤는데 물함량이 낮아질수록 농축물 내의 EPA 및 DHA 함량과 이들의 수율이 증가되는 경향이었다. 한편, 비누화된 용액을 결정화시킬 때의 냉각온도 및 냉각과정은 공정의 효율에 큰 영향을 끼치지 않았다. 무수 에탄올을 사용하고 비누화된 용액을 10℃로 급속 냉각하였을때 EPA와 DHA 함량이 원료 정어리유의 14.2%와 10.7%에서 각각 34.1%와 27.8%로 증가된 PUFA 농축물을 84.5%의 수율로 얻을 수 있었다.