Journal of Chemistry, Vol. 45 (6), P. 757 - 762, 2007

POLYUNSATURATED FATTY ACID ENRICHMENT BY COMPLEXATION WITH SILVER ION

Received 13 October 2006

LAI MAI HUONG

Faculty of Chemical Technology, HCMC University of Technology

SUMMARY

The extraction of methyl esters of tuna oil has been performed, and the extraction behavior is discussed. The effects of $AgNO_3$ concentration, the addition of water-soluble polar solvents such as methanol to aqueous silver nitrate solutions and the presence of organic solvent on the recovery yield of PUFA-ME have been also investigated. Results show that docosahexaenoic acid methyl ester (DHA-ME) and eicosapetaenoic acid methyl ester (EPA-ME) and can be selectively extracted into silver nitrate solutions.

I - INTRODUCTION

Fish oils are a readily available source of polyunsaturated fatty acids (PUFA) which play an important role in human health and nutrition [1, 2]. Eicosapentaenoic acid (EPA), *n*-3 fatty acids and docosahexaenoic acid (DHA) have been shown to be of major importance in the prevention of a number of diseases [3 - 7]. Long-chain PUFAs are now considered as essential for infant growth and development [8, 9]. Due to the health benefits of the *n*-3 fatty acids, there has been a demand from the food, neutraceutical food and pharmaceutical industries for stable, high purity, concentrated *n*-3 products. Several extraction and physical fractionation methods are reported to concentrate *n*-3 fatty acids from marine oils, such as crystallization in solvent [10], high performance liquid chromatography [11] and supercritical fluid extraction [12]. Vacuum distillation requires relatively high temperatures which can result in decomposition of PUFAs. The chromatographic method is unsuitable for treating large amount of PUFAs. Supercritical fluid extraction has a disadvantage that the separation of PUFAs with different degrees of unsaturation with the same carbon number is difficult.

On the other hand, solvent extraction can be operated under mild conditions and is suitable for mass production. Recently it was reported that methyl or ethyl esters of PUFAs such as DHA and EPA could be successfully extracted into silver nitrate solutions since silver ion can complex with carbon-carbon double bonds of PUFAs [13, 14].

In this study, experiments on the extraction of methyl esters of various PUFAs (PUFA-ME) including DHA-ME and EPA-ME presented in tuna oil have been performed, and the extraction behavior is discussed. The effects of $AgNO_3$ concentration, the addition of water-soluble polar solvents such as methanol to aqueous silver nitrate solutions and the presence of organic solvent on the recovery yield of PUFA-ME have been also investigated.

II - MATERIALS AND METHODS

1. Materials and solutions

Tuna oil was obtained from tuna byproducts by wet-rendering method. Tuna oil used in these

experiments has compositions of 29.7% saturated fatty acids (SFA), 19.2% monounsaturated fatty acids (MUFA) and 50.2% PUFA. Methyl esters of fatty acids were prepared according to the methods of Belarbi et al. [15] excepted that methanol was added instead of ethanol.

Tuna methyl esters were dissolved in hexane to obtain a concentration of 20% (w/v). In a series of experiments, this concentration was changed from 13.3%, 20%, 40% and 100%.

Aqueous solutions were prepared by dissolving silver nitrate in deionized water. In a series of experiments, water-soluble alcohol, such as methanol was added to aqueous silver nitrate solutions.

2. Extraction experiments

An oil phase containing a PUFA-MEs and an aqueous phase containing $AgNO_3$ were shaken in a vial for 3 hours, a time period which was confirmed to be adequate for attainment of equilibrium. The volume ratio of aqueous to organic phase was adjusted so that the ratio of tuna methyl esters to the aqueous phase was always 1:5 (w/v). After equilibration, the volume of each phase was measured, and the concentration of PUFA-ME in the oil phase, [PUFA-ME], was determined by a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-8A). The concentration of the Ag.PUFA-ME complex in the aqueous phase, [complex], was calculated by a material balance from the initial concentration and equilibrium concentration of PUFA-MEs in the organic phase and the volumes of each phase. The recovery of PUFA-ME (H) was calculated as the ratio of the amount of PUFA-ME in the aqueous phase to the initial amount of PUFA-ME in the organic phase.

III - RESULTS AND DISCUSSION

1. Effect of $AgNO_3$ concentration

The effect of silver nitrate concentration on the fatty acid profile in the organic and aqueous phase is shown in figures 1 and 2, respectively. *n*-hexane was used as the solvent and the initial concentration of total tuna methyl esters in hexane was 20% (w/v).

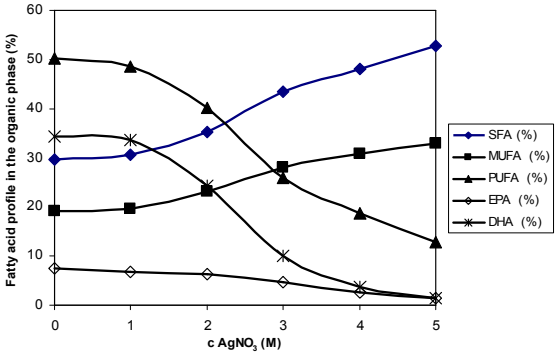

Figure 1: Effect of silver nitrate solution on fatty acid profile in organic phase

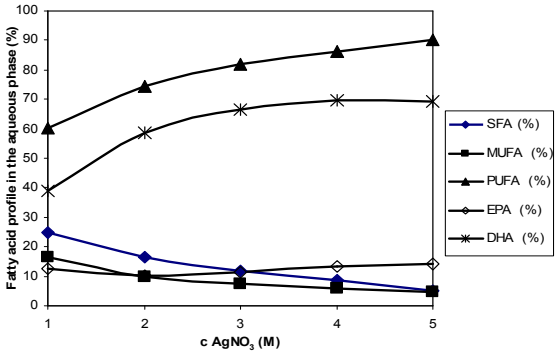

Figure 2: Effect of silver nitrate solution on fatty acid profile in aqueous phase

As can be seen from the figures 1 and 2, percentages of SFA-ME and MUFA-ME increased in the organic phase and decreased in the aqueous phase when silver nitrate concentration increased. On the other hand, PUFA-ME, DHA-ME and EPA-ME concentration had inversed trend.

It is clear that PUFA-ME with a high degree of unsaturation such as DHA-ME and EPA-ME can be selectively extracted in the aqueous solutions. The same results were obtained by other authors [16] and can be explained by the following extraction mechanism: First, PUFA-ME dissolves physically in the aqueous phase. Then, the dissolved PUFA-ME forms a complex with Ag^+ in the aqueous phase.

$$(PUFA\text{-}ME)_0 \leftrightarrow (PUFA\text{-}ME)_w,$$

$$K_D = [PUFA\text{-}ME]_w/[PUFA\text{-}ME]_0 \qquad (1)$$

$$(PUFA\text{-}ME)_W + nAg \leftrightarrow (PUFA\text{-}ME.nAg)^{n+}{}_W = [(PUFA\text{-}ME.nAg)^{n+}{}_w / \{[PUFA\text{-}ME]_w . [Ag^+]_w{}^n\} \quad (2)$$

Here, K_D is the partition coefficient, is the stability constant of complex formation, and PUFA-ME.nAg^{n+} is the complex.

The partition coefficient K_D is depended on the polarity of PUFA-ME and that the logarithm of the partition coefficient of fatty acid methyl or ethyl esters between organic phase and water decreases linearly with the carbon number [17]. On the other hand, the stability constant of complex formation is depended on the number of double bonds. The values of SFA-ME and MUFA-ME are negligible so only K_D plays a role in extraction mechanism. However, because SFA-ME and MUFA-ME are highly non-polar, these values are also small, which resulted in small concentrations of SFA-ME and MUFA-ME in the aqueous phase. By contrast, with the same carbon number, K_D and β of a PUFA-ME are always higher than of SFA-ME or MUFA-ME and β values of PUFA-ME get bigger when the concentration of $AgNO_3$ increases. As a result, concentrations of PUFA-ME in the aqueous phase increase when the concentration of $AgNO_3$ increases. The effect of $AgNO_3$ concentration on the recovery H of EPA-ME and DHA-ME is shown in figure 3.

Results shown in figure 3 once confirms that highly unsaturated PUFA-ME as EPA-ME and DHA-ME can be selectively extracted in the aqueous solutions. When the concentration of $AgNO_3$ increased up to 5 M, the recovery of EPA-ME and DHA-ME in the aqueous phase reached over 90%. EPA differs from DHA in the group of $–CH=CH_2$. It was reported that when hydrogen in a hydrocarbon RH is substituted by $-CH=CH_2$, the partition coefficient K_D between organic phase and water decreases by a factor of 5.4 [18]. Therefore, K_D (DHA-ME) is supposed to be lower than K_D(EPA-ME). As shown in Figure 3, however, the recovery H of DHA-ME is higher than that of EPA-ME. This fact also suggests that the ability of PUFA-ME to form a complex with silver ion has a decisive effect on H.

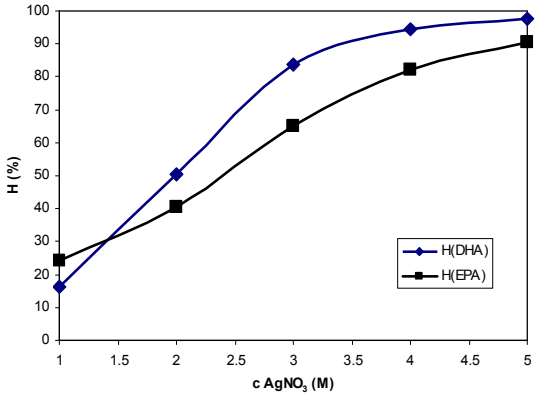

Figure 3: Effect of $AgNO_3$ concentration on the recovery H of EPA-ME and DHA-ME

2. Effect of addition of polar solvent to the aqueous phase

Figures 4 and 5 show the effect of addition of various amounts of methanol to the aqueous phase on the fatty acid profiles obtained in both phases. $AgNO_3$ concentration was remained at 2 mol/l. It was shown that at lower $AgNO_3$ concentration, there was only small effect of addition of methanol on the PUFA extraction ability (percentage and recovery of PUFA) (results not shown).

It can be seen that in both phases, the percentage of what ever fatty acid has inverse tendencies. When methanol concentration in the aqueous phase increased, the percentage of SFA-ME and MUFA-ME increased in the organic phase but decreased in the aqueous phase. On the other hand, the percentage of PUFA-ME, DHA-ME and EPA-ME decreased in the organic phase and increased in the aqueous phase when methanol concentration in the aqueous phase goes up. At the highest methanol concentration, the percentage of PUFA-ME, EPA-ME and DHA-ME in the aqueous phase reached 93.1, 14.8 and 69.9%, respectively but there were in the organic phase only 1.3% DHA-ME, 0.8% EPA-ME and 10.3% total PUFA-ME.

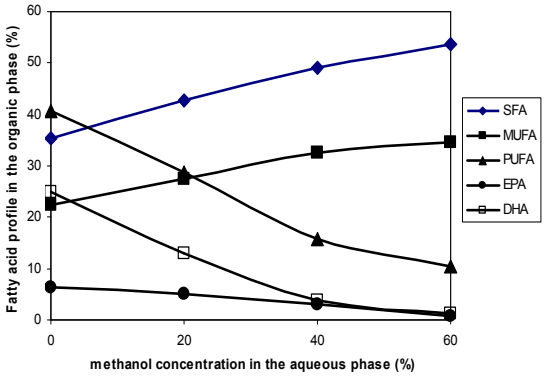

Figure 4: Effect of methanol concentration in the aqueous phase on fatty acid profile in the organic phase. c($AgNO_{3)}$ = 2 M

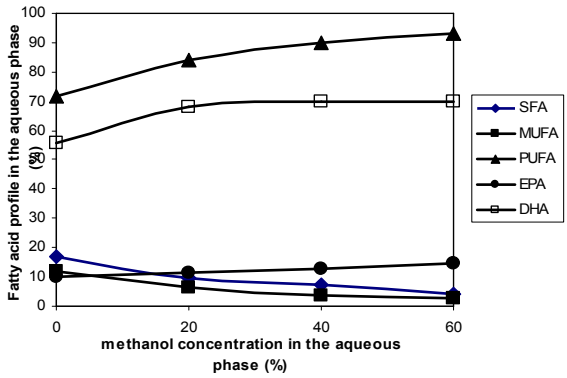

Figure 5: Effect of methanol concentration in the aqueous phase on fatty acid profile in the aqueous phase. c($AgNO_{3)}$ = 2 M

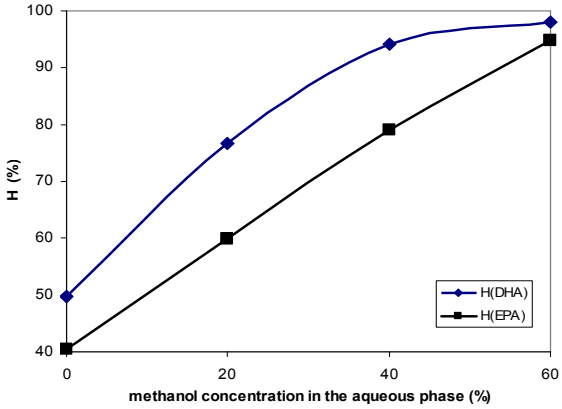

Figure 6: Effect of methanol concentration in the aqueous phase on H_{EPA} and $H_{DHA.}$ c($AgNO_{3)}$ = 2 M

Figure 6 shows effect of methanol addition on the recoveries H_{EPA} and H_{DHA}. H of both EPA and DHA increased remarkably when methanol concentration in the aqueous phase increased. At 0% methanol, H_{EPA} and H_{DHA} were only 49.6% and 40.5% respectively, but at 60% methanol, they reached 98.0% and 94.8% respectively.

As has been explained above, the PUFA-ME extraction mechanism can be described by the equation 1 and 2. When methanol is added to the aqueous phase, there may be changes in coefficients K_D and β. Teramoto et al. [16] measured coefficients K_D and β of EPA when added various methanol volume fractions to the aqueous phase. Their results show that, $K_{EPA\text{-}Et}$ at 25% methanol was $2.8.10^{-7}$ and $V_{EPA\text{-}Et}$ = $3.3.10^{6}$ (l/mol)^5, but at 50% methanol, $K_{EPA\text{-}Et}$ = $1.11.10^{-5}$ and $\beta_{EPA\text{-}Et}$ = $2.6.10^{6}$ (l/mol)^5. It is evidently that K_D increases remarkably but β does not decrease significantly. Since the ability of methanol to complex with Ag^+ was reported to be low [19], the decrease in the free silver ion concentration due to the presence of methanol is small. Therefore, the degree of a decrease in β by addition of methanol is rather small. On the other hand, the value of K_D considerably increases in an increase of the methanol concentration because the hydrophilicity of the aqueous solution decreases. Consequently, H increases by the addition of methanol, as shown in Figure 6.

At 60% methanol, H_{EPA} and H_{DHA} both reached over 90%, nearly 100%. If the methanol concentration is more than 60%, the recoveries H of EPA and DHA would remain stable as can be predicted from the tendency of H_{DHA}. This value remained steady after the methanol concentration reached 40%. On the other hand, $AgNO_3$ solubility is less in methanol than in water. From these reasons, the methanol concentration of 60% seems to be the optimum. When methanol was added to the aqueous 2M $AgNO_3$ phase at 60% concentration, the PUFA-MEs purity and recovery obtained in the aqueous phase were comparable with the case of 5M $AgNO_3$ aqueous solution without methanol.

It is apparently, that methanol can replace significant amount of $AgNO_3$.

Effect of Solvent volume in the Oil Phase. The effect of solvent volume in the oil phase on the extraction equilibriums of PUFA-MEs was investigated. The initial silver nitrate concentration was 2 M and methanol was added to the aqueous phase at the concentration of 60%. The solvent volume used to solubilise FAMEs was adjusted so that the solvent (hexane) in the oil phase to the aqueous phase ratios were changed from 0:1 to 1.5:1 (v/v). Results are shown on figures 7, 8 and 9.

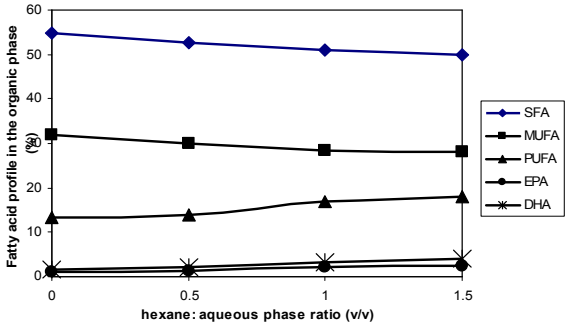

Figure 7: Effect of solvent volume in the oil phase on the fatty acid profile in the organic phase

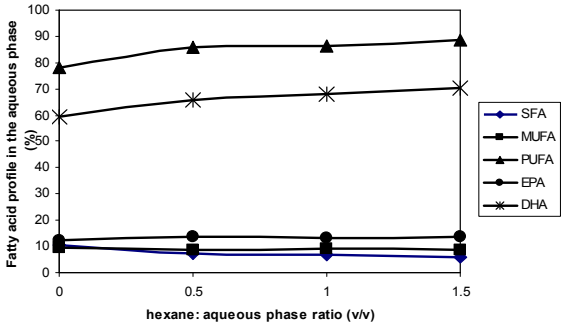

Figure 8: Effect of solvent volume in the oil phase on the fatty acid profile in the aqueous phase

It can be seen that when the hexane to aqueous phase ratio increased, the fatty acid profiles in both phase did not change significantly but H decreased considerably. When the volume of solvent in the oil phase increased but the initial amount of FAMEs was the same, K_D and β were not affected but the initial concentration of PUFA-MEs in the oil phase $[PUFA\text{-}MEs]_o$ decreased. From equations 1 and 2, $[PUFA\text{-}MEs]_w$ would decrease. Other way saying, there would be fewer FAMEs (both saturated and unsaturated) in the aqueous phase, which led to the decrease in the recovery H.

Results in figure 9 suggest that the solvent in the oil phase can be omitted in order to obtain high recovery of EPA and DHA. The extraction process will be more profitable by avoiding of using solvent.

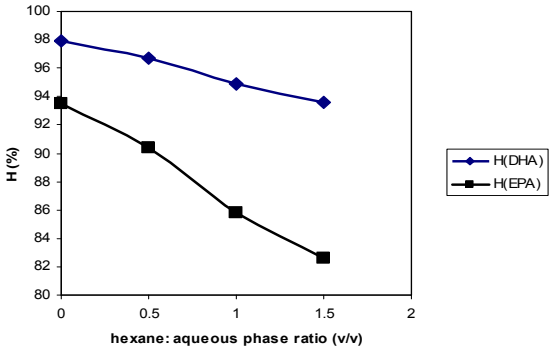

Figure 9: Effect of solvent volume in the oil phase on H_{EPA} and H_{DHA}

IV - CONCLUSIONS

From the experiments on the extraction of mixture of methyl esters of fatty acids obtained from tuna oil into silver nitrate solutions, the following conclusions were obtained:

1. The number of double bonds in PUFA-MEs has a decisive effect on their purity and recovery, and PUFA-MEs with a high degree of unsaturation such as DHA-ME and

EPA-ME can be selectively extracted into silver nitrate solutions.

2. The purity and recovery of PUFA-MEs can be remarkably increased by addition of water-soluble alcohols such as methanol.

3. The solvents in the oil phase can be omitted in order to obtain high recovery and high profitability.

Acknowledgements: *I would like to thank Ing. Tran Thanh Binh for helping in analyzing fatty acid profiles.*

REFERENCES

1. W. Kolanowski, F. Swiderski, S. Berger. Inter. J. Food Sci. Nutri., 50, 39 - 49 (1999).
2. F. Shahidi, U. N. Wanasundara. Trends Food Sci. Technol., 9, 230 - 240 (1998).
3. R. C. Minnis, I. U. Haq, P. R. Jackson, W. W. Yeo, L. E. Ramsay. J. Human Nutr. Dietetics, 11, 13 - 19 (1998).
4. G. S. Rambjor, A. I. Walen, S. L. Windsor, W. S. Harris. Lipids, 31, S45 - 49 (1996).
5. R. G. Ackman. J. Aquatic Food Product Technol., 5, 7 - 26 (1996).
6. D. J. Garcia. Food Technol., 52, 44 - 49 (1998).
7. Y. -Y. Linko, K. Huyakawa. Trends in Food Sci. Technol., 7, 59 - 63 (1996).
8. R. G. Ackman. Lipids 34, 125 - 128 (1999).
9. T. Krawczyk. Inform, 12, 1064 - 1074 (2001).
10. S. R. Moore, G. P. McNeill. J. Am. Oil Chem. Soc., 73, 1409 - 1414 (1996).
11. M. I. Aveldano, M. Van Rollins, L. A. Horrocks. J. Lipid Res., 24, 83 - 93 (1983).
12. W. Bo. Nilsson, Jr. E. J. Gauglitz, K. K. Hudson, V. F. Stout, J. Spinnelli. J. Am. Oil Chem. SOC., 65, 109 - 117 (1988).
13. Y. Nishi, M. Isozaki, Y. Matsuba. Proc. Annu. Meet. SOC. Chem. Eng. Jpn., 56th, 65 (1991a).
14. [14] Nishi, Y.; Nishigaki, H.; Matsuba, Y. Separation of DHA by Liquid Membrane Using Silver Nitrate as Carrier. Roc. Autumn Meet. Soc. Chem. Eng. Jpn. 1991b, 24th, Vol. 2, 252
15. E. H. Belarbi, E. M., Y. Chisti: A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme and Microbial Technology, 26, 516 - 529 (2000).
16. M. Teramoto, H. Matsuyama, N. Ohnishi, S. Uwagawa, and K. Nakai Ind. Eng. Chem. Res., Vol. 33, P. 341 - 345 (1994).
17. R. Smith, C. Tanford. Proc. Natl. Acad. Sci. USA, 70, 289 - 293 (1973).
18. M. Tanaka. Chemistry of Solvent Extraction; Kyoritsu Shuppan: Tokyo, P. 152 (1977).
19. F. R. Hartley, G. W. Searle, R. M. Alcock, D. E. Rogers. J. Chem. SOC., Dalton Trans., 469 - 477 (1977).