Songklanakarin J. Sci. Technol.
38 (2), 163-170, Mar. - Apr. 2016

http://www.sjst.psu.ac.th

Original Article

Concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of Asian catfish oil by urea complexation: optimization of reaction conditions

Pornpisanu Thammapat[1], Sirithon Siriamornpun[2], and Patcharin Raviyan[1]*

[1] *Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100 Thailand.*

[2] *Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham, 44100 Thailand.*

Received: 7 May 2015; Accepted: 1 October 2015

Abstract

Optimization of the concentrating conditions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) extracted from Asian catfish oil was studied to obtain a maximum concentration. The crude fish oil was extracted from the belly flap and adipose tissue of Asian catfish, and the extracted oil was used as fresh crude oil. The EPA and DHA were concentrated by the urea complexation method. A hexagonal rotatable design was applied to examine the effects of crystallization temperature and urea-to-fatty acid ratio on the total content of EPA and DHA (Y_1) and the liquid recovery yield (Y_2). The second order polynomial regression models for Y_1 and Y_2 were employed to generate the response surfaces. Under the optimum conditions of -20 °C and a urea-to-fatty acid ratio of 4 (w/w), the total concentration of EPA and DHA could be increased by up to 88%, while a liquid recovery yield of 26% was obtained.

Keywords: Asian catfish, omega-3 fatty acid, optimization, *Pangasius bocourti*, response surface

1. Introduction

The Asian catfish (*Pangasius bocourti*) is a catfish species of the Pangasiidae family (Orban *et al.*, 2008). This fish is being widely cultured in floating cages in the Mekong River in Northeastern provinces of Thailand. The Asian catfish is popular in Thai dishes and is often farmed for food. Among other farm fishes, it has provided local Thai people with fish protein and fish oil. Recently, it has become a highly demanded fish in the European market and has been exported as frozen fillets to European countries (Cacot *et al.*, 2002; Anonymous, 2005).

* Corresponding author.
Email address: patraviyan@gmail.com

However, many of the Asian catfish by-products are still not utilized, and there are issues associated with acquiring and processing them (Wu and Bechtel, 2008). The ability to process Asian catfish by-products or refrigerate large volumes of by-product remains low, especially at small, remote processing plants. This problem often results in as much as 60-70% of the by-products being discarded. The major by-products from the processing line of Asian catfish in Thailand are the heads, tails, skin, belly flaps, adipose tissue and viscera (Anonymous, 2005; Silva and Dean, 2001).

Fish has been known as a source of high-quality protein and lipid (Weber *et al.*, 2008), with fish oil being an important source of omega-3 polyunsaturated fatty acids (PUFA) (Navarro-García *et al.*, 2004). PUFA takes part in the vascular retina formation and brain development of the child during pregnancy (Gould *et al.*, 2013; San Giovanni and

Chew, 2005; Navarro-García *et al.*, 2004; Simopoulos, 1996; Hoffman and Uauy, 1992). Because PUFA can also reduce blood pressure, signify LDL cholesterol and help preventing several diseases such as thrombosis, hypertension, type 2 diabetes, inflammatory disease, arrhythmia and coronary heart disease, it is essential to the human health (Durrington *et al.*, 2001; Simopoulos, 2002; Weber *et al.*, 2008).

Fish oil containing omega-3 PUFAs has been in high demand for pharmaceutical and dietetic purposes (Jonzo *et al.*, 2000). However, fish oils are unattractive because they contain substantial amounts of undesirable cholesterol and saturated fatty acids (SFA) (Wanasundara and Shahidi, 1999). Some studies indicated that the PUFA concentrates, devoid of more cholesterol and SFA, are much better than marine fish oils themselves since they allow the daily intake of total lipid to be kept as low as possible (Haagsma *et al.*, 1982). Fish oil has been preferentially used as a raw material to prepare EPA and DHA concentrates due to its potential applications in the food and pharmaceutical industries (Gámez-Meza *et al.*, 2003).

The PUFA concentrates can be produced by several methods, including supercritical fluid extraction, freezing crystallization, urea complexation, molecule distillation, silver ion complexation, lipase concentration and high performance liquid chromatography (Shahidi and Wanasundara, 1998; Medina *et al.*, 1998; Liu *et al.*, 2006; Corrêa *et al.*, 2008; Chakraborty and Raj, 2009; Chakraborty *et al.*, 2010). However, the simplest and most efficient technique for obtaining PUFA concentrates in the form of free fatty acids is the urea complexation method (Liu *et al.*, 2006; Wanasundara and Shahidi, 1999; Patil and Nag, 2011). The main application of the urea complexation method is separation of saturated and mono-unsaturated fatty acids from PUFA (Medina *et al.*, 1998; Wanasundara and Shahidi, 1999; Patil, 2014).

Urea complexation has the advantage that the complexed crystals are highly stable. As a result, the filtration does not necessarily have to be carried out at very low temperatures which solvent crystallization of fatty acids would require (Wanasundara and Shahidi, 1999; Liu *et al.*, 2006; Suriani *et al.*, 2014). This technique is also favored by many researchers and fish oil factories because the complexation depends upon their shape, size, geometry and configuration of the fatty acid moieties due to the presence of multiple double bonds, rather than pure physical properties such as melting point or solubility (Shahidi and Wanasundara, 1998; Medina *et al.*, 1998; Wanasundara and Shahidi, 1999; Liu *et al.*, 2006; Patil and Nag, 2011).

Within this study, we aimed at using by-products of Asian catfish fillets, namely the belly flap and adipose tissue, for the production of fish oil. Urea complexation of Asian catfish oil was carried out to concentrate EPA and DHA of oil. Variables such as crystallization temperature (X_1, °C) and urea-to-fatty acid ratio (X_2, w/w) were studied collectively in order to optimize the conditions to obtain a maximum concentration of EPA and DHA.

2. Materials and Methods

2.1 Materials

The belly flap and adipose tissue of the Asian catfish (*P. bocourti)* were obtained from a fish-filleting factory located in Nakhon Phanom Province, Thailand. The extraction, refining (R) and bleaching (B) of the oil were carried out according to recommended procedures for fish oil (Sunarya *et al.*, 1996; Bimbo, 1998). The RB oil was stored under nitrogen at -25°C in amber glass container until used. Fatty acid methyl esters were purchased from either Fluka (Buchs, Switzerland) or Sigma (St. Louis, USA). All other chemicals used in this study were of analytical grade.

2.2 Analysis of fatty acid composition

Fatty acid methyl esters (FAME) of the Asian catfish oil were prepared by transesterication according to the method of Yang *et al.* (2006). In detail, 3 ml HCl–methanol reagent and 1 ml toluene reagent were added to the oil of 100 mg. They were then heated at 70°C for 2 hrs. Fatty acid methyl esters were extracted in 2 ml hexane and stored at -25°C before chemical analysis. The fatty acid methyl esters were analyzed by a SHIMADZU (GC-2014) gas chromatography with a flame ionization detector (FID) (Thammapat *et al.*, 2015).

2.3 Preparation of free fatty acids from Asian catfish oil

The preparation of free fatty acids from Asian catfish oil took place according to the following procedure. A quantity of Asian catfish oil of 175 g was treated with 200 ppm butylated hydroxytoluene (BHT) before saponification with a mixture of 40.25 g KOH, 77 ml distillated water and 462 ml of 95% (v/v) aqueous ethanol. The saponification was operated at 62±2°C for 1 hr under nitrogen. Distilled water of 350 ml was added to the saponified mixture and the unsaponifiable mixture was extracted into hexane (2×200 ml) and discarded. The aqueous layer containing the saponified matter was acidified to pH=1.0 with 3 N HCl. The mixture was transferred to a separating funnel and the liberated fatty acids were extracted into 350 ml hexane. The hexane layer containing free fatty acids was then dried over anhydrous sodium sulfate, and the solvent was removed in a rotator evaporator at 40°C under vacuum to recover free fatty acids which were then stored under nitrogen at -25°C in dark amber glass containers until used in the urea complexation.

2.4 Preparation of EPA and DHA concentrates from Asian catfish oil by urea complexation

The separation of EPA and DHA from the hydrolyzed fatty acid mixture of Asian catfish oil was carried out by urea-fatty acid adduct formation according to the following

procedure. Free fatty acids of 300 g were mixed with 20% (w/v) urea in 95% aqueous ethanol and heated at 60-70°C, with stirring, until the whole mixture turned into a clear homogeneous solution. The urea-to-fatty acid ratio was changed by using different amounts of urea (2:1, 3:1 and 4:1 w/w). Initially, the urea-fatty acid adduct was allowed to crystallize at -5°C, -10°C, -15 °C and -20 °C for 6 hrs. The crystals formed were separated from the liquid by filtration under suction using a Buchner funnel lined with a No.1 Whatman filter paper. The filtrate was diluted with an equal volume of water and was acidified to pH4-5 with 6 N HCl; an equal volume of hexane was subsequently added. The mixture was stirred thoroughly for 1 h and then transferred to a separating funnel. The hexane layer, containing liberated fatty acids, was separated from the aqueous phase. The hexane phase was washed out with distilled water (2×150 ml) to remove any remaining urea and then dried over anhydrous sodium sulfate, and the solvent was removed in a rotator evaporator at 40°C under vacuum. The percentage recovery was calculated.

2.5 Gas chromatography analysis

Free fatty acids were transformed into the corresponding methyl esters (Yang *et al.*, 2006). The fatty acid methyl esters were analyzed by a SHIMADZU (GC-2014) gas chromatography with a flame ionization detector (FID). The esters were separated on a 30 m × 0.25 mm i.d. wall-coated open tubular fused silica capillary column coated with DB-WAX. Column injector and detector temperatures were 250 and 270°C, respectively. The carrier gas was nitrogen flowing at 1.27 ml/min. The temperature program was 150-180°C at 20°C/min, then from 180°C to 220°C at 2.5°C/min, held at 220°C for 3 min, then from 220°C to 230°C at 10°C/min, held at 230°C for 3 min and from 230°C to 235°C at 5.0°C/min, held at 235°C for 10 min. Individual methyl esters were identified against the retention time of standard methyl esters.

2.6 Optimization procedure for production of EPA and DHA concentration by urea complexation of Asian catfish oil

In this study, the hexagonal rotatable design (Gacula and Singh, 1984) was employed to study the responses, such as the total content of EPA and DHA and the liquid recovery yield (Y variables) by urea complexation of Asian catfish oil. The crystallization temperature (X_1) and urea-to-fatty acid ratio (X_2) were independent variables employed to optimize Y variables. The level of variables for the development of the model is represented in Table 1. Triplicate reactions were carried out at all designed points except at the central point (0,0) where four replications were performed to allow the estimation of the 'pure error'. All experiments were carried out in a randomized order to minimize the effect of unexplained variability in the observed responses due to extraneous factors. A quadratic polynomial regression model was assumed for predicting individual *Y* variables. The model proposed for each response of Y is:

$$Y = \beta_0 + \sum \beta i\, X_i + \sum \beta_{ii} X^2_i + \sum\sum \beta_{ij} X_i X_j \quad (1)$$

In this model, β_0, β_i, β_{ii} and β_{ij} were intercept, linear, quadratic and interaction regression coefficient terms, respectively, and X_i and X_j were independent variables. The Design-Expert was used for multiple regression analysis, analysis of variance (ANOVA) and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. Response surfaces were developed using the fitted quadratic polynomial equations obtained from RSREG analysis by holding the independent variables with the least effect on the response at a constant value and by changing the levels of the other variables.

3. Results and Discussion

3.1 Fatty acid composition

The compositions of fatty acids of the Asian catfish oil are shown in Table 2. The predominant fatty acids of Asian catfish were SFA (35.61%), followed by PUFA (37.88%) and MUFA (25.61%). The oleic acid (18:1) was the major MUFA, while palmitic acid (C16:0) and linoleic acid (18:2) were the major SFA and PUFA, respectively. These results are in agreement with previous studies on fatty acids of Asian catfish, which is the largest farmed Thailand native, warm water, fresh fish (Thammapat *et al.*, 2010). The major contributors to *n*-3

Table 1. Variables (factors) used for hexagonal rotatable design.

Coded- variable levels		Natural-variable levels	
Z_1	Z_2	X_1 (Crystallization Temperature, °C)	X_2 (Urea-to-fatty acid ratio, w/w)
1.00	0.00	-5	3.00
0.50	0.866	-10	3.87
-0.50	0.866	-20	3.87
-1.00	0.00	-25	3.00
-0.50	-0.866	-20	2.13
0.50	-0.866	-10	2.13
0.00	0.00	-15	3.00

Table 2. Fatty acid composition (% total fatty acids) of Asian catfish oil.

Fatty acid composition	(% total fatty acids)
C14:0	4.65±0.07
C16:0	22.65±0.18
C16:1*n*-9	1.53±0.05
C18:0	8.31±0.09
C18:1*n*-9	24.98±0.20
C18:2*n*-6	16.53±0.15
C18:3*n*-3	0.75±0.09
C20:3*n*-6	0.82±0.05
C20:4*n*-6	0.85±0.08
C20:5*n*-3	8.17±0.06
C22:*n*-3	10.76±0.11
SFA	35.60±0.19
MUFA	26.51±0.16
PUFA	37.88±0.16
$\sum n$-3 PUFA	20.50±0.13
$\sum n$-6 PUFA	18.20±0.28

Mean values ± standard deviation of determinations for triplicate samples.

PUFA was 22:6 while 18:2 was found to be the major *n*-6 PUFA. Similar results were observed in other fish water fish, such as sardine (Létisse *et al.*, 2006), Lake Superior fish (Wang *et al.*, 1990) and rainbow trout (Haliloðlu *et al.*, 2004)

3.2 Concentration of EPA and DHA of Asian catfish oil

Experimental values obtained for responses. The total content of EPA and DHA and the liquid recovery yield of Asian catfish oil for ten design points are given in Table 3. The maximum increase of total EPA and DHA content was 90.65%, with exhibited 25.42% liquid recovery yield in the non-urea complexed fraction, which was obtained at crystallization temperature of -20°C and urea-to-fatty acid ratio of 3.87.

The crystallization temperature and urea-to-fatty acid ratio were the most influential variables affecting the degree of PUFA concentration. Generally, enrichment of PUFA in concentrate and liquid recovery yield varied inversely with the decreasing crystallization temperature as well as with the increasing urea-to-fatty acid ratio (Liu *et al.*, 2006). At low temperatures, fatty acids had a greater tendency to form urea compounds than at high temperatures, and the urea-to-fatty acid ratio could be used to segregate the fatty acids by their degree of unsaturation. Liu *et al.* (2006) have reported similar results for the urea complexation experiment conducted on tuna oil. In addition, Wanasundara and Shahidi (1999) have reported that DHA was a major portion in the non-urea complexed fraction, while EPA was a major portion in the urea complexed fraction. Urea complexation of seal blubber oil resulted in an increase in the total PUFA content by up to 89.50% in the non-urea complexed fraction.

Urea complexation of Asian catfish oil in this experiment resulted in less EPA content in the non-urea complexed fraction as compared to that of the DHA. This could be because the content of EPA in the Asian catfish was lower than that of DHA. Nevertheless, EPA had a greater tendency than DHA to form urea compounds; this is confirmed by previous studies on marine fish oil (Ratnayake *et al.*, 1988; Wanasundara and Shahidi, 1999).

When urea crystallizes from the mixture, the saturated fatty and mono-unsaturated fatty acids were first included in the crystal while the PUFAs remained in the mixture. The long-chain mono-unsaturated fatty acids, especially those of the C20 and C22, form complexes with urea more readily than those of the shorter chain saturated fatty acids (C14 and C16) (Medina *et al.*, 1998; Shahidi and Wanasundara, 1998). The tendency of fatty acids to combine with urea decreased with

Table 3. Hexagonal rotatable design arrangement and responses for non-urea complexed fraction of the Asian catfish.

Variable levels		Response, Y(non-urea complexed fraction)	
X_1 (Crystallization Temperature, °C)	X_2(Urea-to-fatty acid ratio, w/w)	Total EPA and DHA(%)	Yield (%)
-5	3.00	65.88±1.32	43.04±0.82
-10	3.87	83.67±2.04	38.96±1.08
-20	3.87	90.65±2.16	25.42±0.65
-25	3.00	74.16±1.83	24.59±0.98
-20	2.13	84.47±1.75	34.07±1.12
-10	2.13	76.67±0.97	41.01±1.25
-15	3.00	83.46±1.38	33.33±1.04
-15	3.00	81.79±2.01	33.78±0.95
-15	3.00	83.23±1.94	33.06±1.14
-15	3.00	81.94±1.56	33.97±0.83

unsaturation increase and with chain-length decrease (Medina *et al.*, 1998). Complete removal of saturated fatty acids by urea complexation could be quite impractical. This was because some saturated fatty acids did not form complex with urea during crystallization time (Liu *et al.*, 2006).

In our present study, it was observed that the urea-to-fatty acid ratio and temperature, both of which are strongly related, are the most influential variables affecting the degree of PUFA concentration. The variation in EPA and DHA concentration with the urea-to-fatty acid ratio is different when the crystallization temperature is low than when it is high (Medina *et al.*, 1998).

3.3 Analysis of model

Optimization of processing conditions, such as crystallization temperature (X_1) and urea-to-fatty acid ratio (X_2), to maximize the total content of EPA and DHA in the prepared concentrate was determined. The multiple regression coefficients obtained by employing a least square technique to predict a quadratic polynomial model for the total content of EPA and DHA (Y_1) and the liquid recovery yield (Y_2).

Examination of these coefficients with the t-test, for the total content of EPA and DHA, indicated that linear and quadratic terms of crystallization temperature and urea-to-fatty acid ratio were highly significant (P<0.01). For the liquid recovery yield, the linear terms of crystallization temperature and urea-to-fatty acid ratio were highly significant (P<0.01) and the quadratic term were also significant (P<0.05). These results suggested that the linear and/or the quadratic effect of crystallization temperature and urea-to-fatty acid ratio may be the primarily determining factors for both EPA and DHA total contents, as well as for the liquid recovery yield. The coefficients of independent variables determined for the quadratic polynomial models for the total content of EPA and DHA (Y_1) and the liquid recovery yield (Y_2) of the prepared concentrate are given below:

$$Y_1 = 82.73 - 5.67Z_1 + 2.85Z_2 - 11.21Z_1^2 + 6.15Z_2^2 - 1.09Z_{12} \quad (2)$$

$$Y_2 = 32.96 + 10.56Z_1 - 4.13Z_2 + 2.36Z_1^2 + 1.76Z_2^2 + 1.73Z_{12} \quad (3)$$

These results show that the models predicted for Y_1 and Y_2 were adequate as indicated by error analysis that showed non-significant lack-of-fit (P>0.05). The adjusted correlation coefficients for the determination (R^2) of the total content of EPA and DHA and the liquid recovery yield were 0.98 and 0.99, respectively.

3.4 Optimization of process conditions to maximize contents of the total content of EPA and DHA and the liquid recovery yield of Asian catfish oil concentrate

The response surfaces for the total content of EPA and DHA and the liquid recovery yield are given in Figure 1 and 2, respectively. The results of canonical response surfaces were given in Table 4. The stationary points for the total content of EPA and DHA by urea complexation predicted a maximum increase of 90.71% at the crystallization temperature of -19.73°C and the urea-to-fatty acid ratio of 3.89. The result of the three-dimensional response surface indicated that the total content of EPA and DHA would be increased with decrease of the liquid recovery yield. Therefore, in the case of a high level of the total content of EPA and DHA, a low liquid recovery yield in concentrate would be desired, and a crystallization temperature of -20.93°C and a urea-to-fatty acid ratio of 3.91 may be suitable.

Previous studies have reported different variable processing conditions for producing fish oil concentrates. For example, Wanasundara and Shahidi (1999) have reported that the content of total ω3 fatty acids was highest at the crystallization temperature of -10°C, urea-to-fatty acid ratio of 4.5 and crystallization time of 24 hrs. On the other hand, Liu *et al.* (2006) have reported that the total content of EPA and DHA was highest at the crystallization temperature of -3.88°C, urea-to-fatty acid ratio of 15.78 (mole ratio) and crystallization time of 15.86 hrs. Our study used a lower

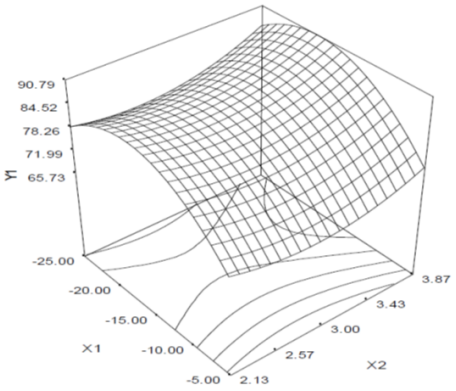

Figure 1. Response surface for the effect of crystallization and urea-to-fatty acid ratio on the total content of EPA and DHA of the concentrate of Asian catfish oil.

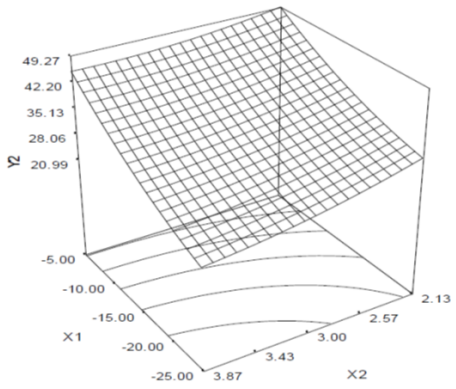

Figure 2. Response surface for the effect of crystallization and urea-to-fatty acid ratio on the liquid recovery yield of the concentrate of Asian catfish oil.

Table 4. Predicted and observed values for response variables in urea complexation experiment of Asian catfish oil.

Response variables	Critical values of independent variables		Stationary point	Predicted value (%)	Observed value[a] (%)
	Crystallization Temperature (°C)	Urea-to-fatty acid ratio (w/w)			
Total EPA and DHA (%)	-19.73	3.89	Maximum	90.71	88.24±1.34
liquid recovery yield	-20.93	3.91	Minimum	24.27	26.42±0.49

[a] Mean values ± standard deviation of determinations for triplicate samples.

crystallization temperature than those used in these studies, because of the higher short chain SFA and long chain MUFA content in Asian catfish oil. It would be difficult to remove those fatty acids from the mixture as they were included in the urea crystals (Medina *et al.*, 1998). Accordingly, the specific crystallization temperature of any individual fish oil should be determined. The crystallization temperature is also dependent on the degree of concentration desired (Shahidi and Wanasundara, 1998).

The adequacy of the models predicted was examined by performing independent experiments at the optimal conditions for both the total EPA and DHA content and the liquid recovery yield. Verification results revealed that the predicted values from these models were reasonably close to the observed ones (Table 4), indicating that the models are suitable for the prediction of the study responses. The urea-to-fatty acid ratio may be used to segregate the fatty acids by their degree of unsaturation. When insufficient amounts of urea are used, fatty acids compete among themselves for complexing with urea; hence fractionation may occur according to the different competing tendencies of the different fatty acids (Medina *et al.*, 1998). As predicted values of optimum crystallization temperature and the urea-to-fatty acid ratio were -19.73 and 3.89% (Table 4), respectively. For practical use, we decided to further study crystallization temperature at -20°C and the urea-to-fatty acid ratio of 4% (w/w) and the results are show in Table 5. The results show that a urea-to-fatty acid ratio of 4, the SFA and MUFA are eliminated while the PUFA remains in solution. The tendency to form urea compounds increases with decreasing crystallization temperature of -20°C. On the other hand, the higher the crystallization temperature, the lower is the tendency of fatty acids to form urea compounds (Medina *et al.*, 1998). According to our study (Table 4 and 5), it has been confirmed the model proposed for the total content of EPA and DHA and liquid recovery yield provided a good prediction for concentrated PUFA production.

Table 5. Fatty acid composition (% total fatty acids) of non-urea complexed fraction obtained at -20°C and a urea-to-fatty acid ratio of 4 (w/w).

Fatty acid composition	(% total fatty acids)
C14:0	0.05±0.01
C16:0	0.24±0.04
C16:1*n*-9	0.03±0.01
C18:0	0.11±0.03
C18:1*n*-9	0.23±0.05
C18:2*n*-6	9.07±0.74
C18:3*n*-3	0.95±0.12
C20:3*n*-6	0.99±0.15
C20:4*n*-6	1.02±0.20
C20:5*n*-3	31.06±1.78
C22:*n*-3	56.25±2.08
$\sum n$-3 PUFA	88.26±2.64
$\sum n$-6 PUFA	11.08±0.70

Mean values ± standard deviation of determinations for triplicate samples.

4. Conclusions

The processing parameters were found to be: a crystallization temperature of -20°C and a urea-to-fatty acid ratio of 4 (w/w). We selected these conditions in order to achieve the highest levels of the total content of EPA and DHA and low liquid recovery yield in concentrate. Under these conditions, the total content of EPA and DHA could be increased by up to 88% with a liquid recovery yield of 26% of the weight of the original Asian catfish oil. The urea complexation was demonstrated to be a feasible method for the concentration of EPA and DHA from Asia catfish oil in food industry.

References

Anonymous. 2005. Pangasius: A new export fish. Food Journal. Thailand. 8, 34-45.

Bimbo, A.P. 1998. Guidelines for characterizing food-grade fish oil. International News on Fats, Oils and Related Materials. 9, 473-483.

Cacot, P., Legendre, M., Dan, T.Q., Tung, L.T., Liem, P.T., Mariojouls, C. and Lazard, J. 2002. Induced ovulation

of *Pangasius bocourti* (Sauvage, 1880) with a progressive hCG treatment. Aquaculture. 213, 199-206.

Chakraborty, K. and Raj, R.P. 2009. Selective enrichment of n-3 polyunsaturated fatty acids with C_{18}-C_{20} acyl chain length from sardine oil using *Pseudomonas fluorescens* MTCC 2421 lipase. Food Chemistry. 114, 142-150.

Chakraborty, K., Vijayagopal, P., Chakraborty, R.D. and Vijayan, K.K. 2010. Preparation of eicosapentaenoic acid concentrates from sardine oil by *Bacillus circulans* lipase. Food Chemistry. 120, 433-442.

Corrêa, A.P.A., Peixoto, C,A., Gonçalves, L.A.G. and Cabral, F.A. 2008. Fractionation of fish oil with supercritical carbon dioxide. Journal of Food Engineering. 88, 381-387.

Durrington, P.N., Bhatnagar, D., Mackness, M.I., Morgan, J., Julier, K., Khan, M.A. and France, M. 2001. An omega-3 polyunsaturated fatty acid concentrate administered for one year decreased triglycerides in simvastatin treated patients with coronary heart disease and persisting hypertriglyceridaemia. Heart. 85, 544-548.

Gacula, M.C.Jr. and Singh, J. 1984. Statistical Methods in Food and Consumer Research, Academic Press, Florida, U.S.A., pp. 113-147.

Gámez-Meza, N., Noriega-Rodríguez, J.A., Medina-Juárez, L.A., Ortega-García, J., Monroy-Rivera, J., Toro-Vázquez, F.J., García, H.S. and Angulo-Guerrero, O. 2003. Concentration of eicosapentaenoic acid and docosahexaenoic acid from fish oil by hydrolysis and urea complexation. Food Research International. 36, 721-727.

Gould, J.F., Smithers, L.G. and Makrides, M. 2013. The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition. 97, 531-544.

Haagsma, N., Gent, C.M., Luten, J.B., Jong, R.W. and Doorn, E. 1982. Preparation of a ω-3 fatty acid concentrate from cod liver oil. Journal of the American Oil Chemists' Society. 59, 117-118.

Hoffman, D.R. and Uauy, R. 1992. Essential of dietary omega-3 fatty acids for premature infants: Plasma and red blood cell fatty acid composition. Lipids. 27, 886-894.

Haliloðlu, H.I., Bayýr, A., Sirkecioðlu, A.N., Aras, N.M. and Atamanalp, M. 2004. Comparison of fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*) living in seawater and freshwater. Food Chemistry. 86, 55-59.

Jonzo, M.D., Hiol, A., Zagol, I., Druet, D. and Comeau, L. 2000. Concentrates of DHA from fish oil by selective esterification of cholesterol by immobilized isoforms of lipase from *Candida rugosa.* Enzyme and Microbial Technology. 27, 443-450.

Létisse, M., Roziéres, M., Hiol, A., Sergent, M. and Comeau, L. 2006. Enrichment of EPA and DHA from sardine by supercritical uid extraction without organic modier I. Optimization of extraction conditions. Journal of Supercritical Fluids. 38, 27-36.

Liu, S., Zhang, C., Hong, P. and Ji, H. 2006. Concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) of tuna oil by urea complexation: optimization of process parameters. Journal of Food Engineering. 73, 203-209.

Medina, A.R., Grima, E.M. Giménez, A.G. and González, M.J.I. 1998. Downstream processing of algal polyunsaturated fatty acids. Biotechnology Advances. 3, 517-580.

Navarro-García, G., Pacheco-Aguilar, R., Bringas-Alvarado, L. and Ortega-García, J. 2004.Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays. Food Chemistry. 87, 89-96.

Orban, E., Nevigato, T., Lena, G.D., Masci, M., Casini, I., Gambelli, L. and Caproni, R. 2008. New trends in the seafood market. Sutchi catfish (*Pangasius hypophthalmus*) fillets from Vietnam: Nutritional quality and safety aspects. Food Chemistry. 110, 383-389.

Patil, D. 2014. Recent trends in production of polyunsaturated fatty acids (PUFA) Concentrates. Journal of Food Research and Technology. 2, 15-23.

Patil, D. and Nag, A. 2011. Production of PUFA Concentrates from poultry and fish processing waste. Journal of the American Oil Chemists' Society. 88, 589-593.

Ratnayake, W.M.N., Olsson, B., Matthews, D. and Ackman, R.G. 1988. Preparation of omega-3 PUFA concentrates from fish oils via urea complexation. European Journal of Lipid Science and Technology. 90, 381-386.

San Giovanni, J.P. and Chew, E.Y. 2005. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research. 24, 87-138.

Shahidi, F. and Wanasundara, U.N. 1998. Omega-3 fatty acid concentrates: nutritional aspects and production technologies. Trends in Food Science and Technology. 9, 230-240.

Silva, J.L. and Dean, S. 2001. Processed catfish product forms, packaging, yields and product mix. South Region Aquaculture Center Publications. No. 184.

Simopoulos, A.P. 1996. The role of fatty acids in gene expression: health implications. Annals of Nutrition and Metabolism. 40, 303-311.

Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy. 56, 365-379.

Sunarya, H., Hole, M. and Taylor, K.D.A. 1996. Methods of extraction composition and stability of vitamin A and other components in dogfish (*Squalus acanthias*) liver oil. Food Chemistry. 55, 215-220.

Suriani, N.W., Lawalata, H.J. and Komansilan, A. 2014. Urea crystallization on the concentrate making of omega-3 fatty acid from oil of tuna fish (*Thunnus Sp*) canning byproduct. International Journal of PharmTech

Research. 6, 1981-1990.

Thammapat, P., Raviyan, P. and Siriamornpun, S. 2010. Proximate and fatty acids composition of the muscles and viscera of Asian catsh (*Pangasius bocourti*). Food Chemistry. 122, 223-227.

Thammapat, P., Meeso, N. and Siriamornpun, S. 2015. Effects of NaCl and soaking temperature on the phenolic compounds, α-tocopherol, γ-oryzanol and fatty acids of glutinous rice. Food Chemistry. 175, 218-224.

Wanasundara, U.N. and Shahidi, F. 1999. Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. Food Chemistry. 65, 41-49.

Wang, Y.J., Miller, L.A., Perren, M. and Addis, P.B. 1990. Omega-3 fatty acids in Lake Superior fish. Journal of Food Science. 55, 71-73.

Weber, J., Bochi, V.C., Ribeiro, C.P., Victório, A.M. and Emanuelli, T. 2008. Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chemistry. 106, 140-146.

Wu, T.H. and Bechtel, P.J. 2008. Salmon by-product storage and oil extraction. Food Chemistry. 111, 868-871.

Yang, L.F., Sirithon, S. and Li, D. 2006. Polyunsaturated fatty acid content of edible insects in Thailand. Journal of Food Lipids. 13(3), 277-285.