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Research Article

Optimizing Conditions for the Purification of Omega-3 Fatty Acids from the By-product of Tuna Canning Processing

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Abstract: This research studied the optimization conditions for separation and purification of omega-3 (ω-3) fatty acids from the by-product of tuna canning processing by urea crystallization. Crystallization reaction conditions of urea inclusion (urea to fatty acid ratio (X_1) and crystallization time (X_2)) were optimized using the Response Surface Methodology (RSM) and a model was developed. Optimization results showed a quadratic polynomial regression equation of $Y = 24.44X_1 + 5.65X_2 - 8.71X_1^2 - 0.19X_2^2 + 1.171X_1X_2 - 12.95$. The maximum response was obtained at an urea to fatty acid ratio of 2.99:1 and a crystallization time of 23.64 h and predicted response of 90.44%. Analysis of variance showed that the urea to fatty acid ratio and crystallization time affected the response. Verification under optimal conditions showed that the purity of ω-3 fatty acids was 89.64% and the enrichment was 2.85 fold. Verification result revealed that the predicted value from this model was reasonably close to the experimentally observed value. The urea crystallization process changed oil quality parameters including oxidation level (peroxide, anisidin, and totox values), Fe, Cu and P concentrations and moisture content and this were mostly due to the saponification process before urea crystallization.

Keywords: By product, concentrate, omega-3 fatty acid, RSM, tuna oil, urea crystallization

INTRODUCTION

Omega-3 fatty acids are one of the fatty acid groups that are known to have health benefits (Basu *et al*., 2006; Connor and Connor, 2007). The major sources of these fatty acids are fish oil and various methods have been developed to enrich fish oil by this group of fatty acids. High ω-3 fatty acids level of fish oil is obtained by many methods such as low temperature solvent crystallization (Ahmadi, 2006), rapid solidification (Moffat *et al*., 1993; Estiasih *et al*., 2005, 2006), enzymatic process (Sridhar and Lakhsminarayana, 1992; Yamane *et al*., 1992; Carvalho *et al*., 2002; Lee *et al*., 2003) and urea crystallization (Haagsma *et al*., 1982; Ackman *et al*., 1988; Ganga *et al*., 1998; Wanasundara and Shahidi, 1999; Hwang and Liang, 2001; Liu *et al*., 2006).

Among the various developed methods, the urea crystallization technique is the most simple and efficient process. The ω-3 fatty acid concentrate produced by this method is in the form of free fatty acids. This technique is well recognized to remove saturated fatty acids and monoenoic fatty acids from oil (Liu *et al*., 2006). Crystallization is an important process for purification and separation. Different crystallization condition can lead to the different crystal formation and influence purification and separation process (Engkvist *et al*., 2000). Urea has capability to form crystal with unsaturated fatty acid due to the different geometric structure between unsaturated and saturated fatty acids. Branched fatty acids or fatty acids that have kinks on their structure have no capability for urea complex formation (Stout *et al*., 1990).

The urea crystallization is an effective technique for PUFA (Polyunsaturated Fatty Acid) concentration and is a method favored by researchers to separate fatty acids based on molecular structure (Fei *et al*., 2010). This technique is able to remove saturated and monounsaturated fatty acids (Wu *et al*., 2008). Saturated and unsaturated fatty acids can be separated by urea crystallization due to the difference of alkyl chain linearity. Saturated fatty acids have straight alkyl chain, whereas unsaturated fatty acids have kinks in their structure, particularly for *cis* (Guil-Guerrero and Belarbi, 2001; Hayes, 2002). In the urea inclusion complex, urea molecules form hydrogen bonds and a parallel tunnel. The urea tunnel structure is stable if the tunnel is filled by a guest compound in close order.

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This tunnel has a diamater based on van der Waals bond radius that varies from 5, 5 to 5, 8 A°. Only specific molecules can be a guest compound to the form urea inclusion complex and the compound exhibits hexagonal structure with guest compounds such as linear hydrocarbon with C atoms totalling more than 6, therefore branched molecules can be separated from linear ones (Yeo and Harris, 1999). Several factors affecting urea-fatty acid crystallization are crystallization temperature, urea to fatty acid ratio and crystallization time. These factors affect the urea-fatty acid inclusion complex formation, therefore influence ω-3 fatty acids levels (Wanasundara and Shahidi, 1999; Hwang and Liang, 2001).

Among various sources of fish oil, the oil from the by-product of tuna (*Thunnus* sp.,) canning processing has not yet been explored as a source of ω-3 fatty acids. This oil is produced in the precooking step in the canning processing. The by-product stick water or liquor contains both oil and water. After sedimentation, the oil can be separated from water and further processed for animal feed. This oil is a rich source of ω-3 fatty acids.

Previous study (Liu *et al*., 2006) using the oil from tuna head showed that the optimum condition for obtaining a high level of ω-3 fatty acids was an urea to fatty acid ratio of 15 (mole/mole), a crystallization temperature of -5°C and a crystallization time of 20 h. The best result of urea crystallization process from various crystallization temperature and urea to fatty acid ratio produced EPA+DHA level of oil of only 46% (Elizabeth, 1992). In this case, urea crystallization was not in optimum conditions, therefore it was urgent to define the optimum condition for urea crystallization.

The Response Surface Methodology (RSM) approach can simultaneously and efficiently define optimum processing conditions for several factors. A one factor-one time method does not describe the true changes due to the fact that simultaneous interactions among factors are not considered. If there are many factors and the interactions influence response, response surface methodology is an appropriate tool for optimizing response and process (Wanasundara and Shahidi, 1999). Many studies aiming to optimize ω-3 fatty acids concentrate preparation have used RSM, including optimizing urea crystallization process (Wanasundara and Shahidi, 1999; Liu *et al*., 2006; Wu *et al*., 2008), but it is not reported yet for fish oil from the by-product of tuna canning processing. The optimization by response surface methodology is faster and more economic in data collection compared to one variable-one time approach or factorial design (Liu *et al*., 2006). Therefore it is important to determine optimum condition for the urea crystallization process in purification of ω-3 fatty acids from fish oil. In this study, the optimizing factors were urea to fatty acid ratio and crystallization time.

MATERIALS AND METHODS

Materials: The material used was fish oil from the by-product of tuna (*Thunnus* sp.,) caning processing that obtained from a fish canning industry at Muncar, East Java, Indonesia. All solvents used for ω-3 fatty acids preparation were technical grade and for analysis were analytical grade including fatty acid methy ester standard mixture and C19:0 methyl ester as internal standard (Sigma Chem. Co., USA).

Preparation of ω-3 fatty acid concentrate: The preparation of ω-3 fatty acid concentrate was done using the method of Haagsma *et al*. (1982) as follows. The saponification was done by mixing 1 kg of fish oil from by product of canning processing with 2 L of NaOH solution in aquadest/EtOH and stirred for 30 min at 60°C. After saponification, the mixture was added by 400 mL aquadest. NaOH solution was prepared by solubilizing 480 g NaOH and 5 g Na_2EDTA in 1.6 L aquadest. Ethanol of 1.6 L was added to that solution. Four L hexane was added and the mixture was stirred for 1 h to extract fatty acids. The upper layer contained unsaponifiable matter was removed. HCl was added to the lower layer and it was stirred until achieving a pH value of 1. Two layers were formed and the lower layer was removed and the upper layer (hexane layer) was evaporated by rotary vaccuum evaporator at 30°C. The fatty acid extract was added to hot urea solution (temperature of 60-65°C) in methanol and agitated at a constant rate. The amount of methanol added to 25 g fish oil was 200 mL. At this stage, the optimization of the urea to fatty acid ratio (Table 1) was performed at 10°C. Sometimes, heating was done to have clear solution. Urea let to the formation of crystal at various times according to the treatments in the response surface methodology design (Table 1). At this stage, crystallization time was optimized. Urea crystals were separated from the mother liquor by filtration and the ω-3 fatty acids in the filtrate was extracted. One litre hexane and 0.5 L concentrated HCl were added to every 3 L filtrate. The mixture was agitated for 1 h. The hexane layer was separated. The amount of aquadest of 1.5 L was added to the lower layer. This layer was further extracted by 1 L hexane. Both hexane extracts were mixed and hexane was removed using rotary vaccuum evaporator at 30°C to obtain fatty acids.

Optimizing by response surface methodology: The optimization technique using response surface methodology was performed in this study. The objective of optimization was to obtain ω-3 fatty acid concentrate with the highest level of EPA

Table 1: Second order model of central composite design for predicting response of EPA+DHA level

	Coded variable		Uncoded variable		
No	X1	X2	Ratio of urea to fatty acid	Crystallization time (hours)	Response EPA+DHA level (% w/w)
1	-1	-1	2.5:1	18	84.44
2	-1	1	2.5:1	30	68.08
3	1	-1	3.5:1	18	76.63
4	1	1	3.5:1	30	73.16
5	0	0	3:1	24	89.16
6	0	0	3:1	24	90.33
7	0	0	3:1	24	89.97
8	0	0	3:1	24	92.08
9	0	0	3:1	24	90.58
10	-1.414	0	2.293:1	24	85.03
11	1.414	0	3.707:1	24	86.99
12	0	-1.414	3:1	15.516	79.76
13	0	1.414	3:1	31.07	73.07

Table 2: Characteristics of fish oil from by-product of tuna canning processing, fatty acid extract (intermediate product) and ω-3 fatty acids concentrate at optimum condition

Quality parameters	IFOMA standard*	Fish oil	Fatty acid extract	ω-3 fatty acids concentrate
Free fatty acid content, % oleat	1-7	3.2500	n.a.	n.a.
Moisture and impurity, %	0.5-1	n.d.	n.a.	n.d.
Peroxide value (meq/kg)	3-20	2.1000	3.55	2.7700
Anisidin value	6-40	28	19.68	9.8800
Totox value	10-60	32.2000	26.80	14.2200
Color, gardner	<14	n.a.	n.a.	n.a.
Color (lovibond)	-	250	n.a.	1.8
Fe, ppm	0.5-0.7	n.d.	n.a.	0.0118
Cu, ppm	<0.3	0.0082	n.a.	0.2990
P, ppm	5-100	0	n.a.	0.0038

*: IFOMA = International Fish meal and Oil Manufacturers Association; n.a.: Not applicable; n.d.: Not detected

(eicosapentaenoic acid) and DHA (docosahexaenoic acid) as the main health beneficial ω-3 fatty acids. Elucidated factors were the urea to fatty acid ratio (w/w) (X_1) and the crystallization time (hours) (X_2) suited to Order 2 Central Composite Design. The treatments were replicated twice. Based on that design, the replication was performed twice for all combined treatments except at central point (0, 0) that performed 5 times. All combined treatments was done randomly to minimize bias. A polynomial quadratic model assumed to predict response could be explained by the equation as follows:

$$Y = \beta_o + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\Sigma\beta_{ij} X_i X_j$$

β_o, β_i, β_{ii}, β_{ij} are intercept, linear regression coefficient, quadratic regression coefficient and interaction, respectively. Whereas X_i and X_j were independent variables. Obtained data was analyzed using Design Expert DX 6.0.10 (trial version) program. Statistical analysis was used to analyze regression or fitted model, analysis of variance and canonical analysis. Response surface and the contour was obtained from the fitted model.

Characterization of fish oil, fatty acid extract and ω-3 fatty acids concentrate from optimum condition: The fish oil from by-product of tuna canning processing, the fatty acid extract (intermediate product) and the ω-3 fatty acids concentrate at optimum condition, some were analyzed according to the food grade fish oil standard from the International Association of Fishmeal and Oil Manufacturers (IFOMA) (Bimbo, 1998) as shown in Table 2.

Analyses included fatty acid composition (methylation method according to Park and Goins (1994) and quantification was performed based on weight percentage using an internal standard of C19:0 methyl ester; oxidation level indicated by peroxide value according to Hiels and Thiel modified by Chapman and Mackey (Adnan, 1980), anisidin and totox value (IUPAC, 1979) and moisture content (AOCS, 1989).

Fatty acid analysis: Fatty acid profile of ω-3 fatty acids was analyzed using gas chromatography (Shimadzu GC 8A). The column used for separation was capillary CBP20 0.25 μm bonded silica column with dimension of 50 mm in length, i.d. 0.22 mm and o.d. 0.33 mm. Nitrogen was used as a gas carrier with a pressure of 200 kg/m^2, while for supporting and burning gas, air and hydrogen was used with a pressure of 0.15 and 0.6 kg/cm^2, respectively. Injector, column and detector temperature was 230, 250 and 230°C, respectively. Samples and standard were injected at the volume of 2 μL.

Table 3: Fatty acid composition of tuna oil from by-product of canning processing and ω-3 fatty acid concentrate at optimum condition

Fatty acid	Fish oil % (w/w)	ω-3 fatty acids concentrate % (w/w)	Degree of enrichment % (w/w)
C14:0	3.06	0.90	
C16:0	17.37	0.05	
C16:1ω-9	0.94	3.11	
C18:0	5.00	0	
C18:1ω-9	12.69	0	
C18:2ω-6	0.71	1.73	
C18:3ω-3	1.11	1.83	
C20:0	0.07	0	
C22:1ω-9	1.88	0	
EPA	6.03	18.48	3.06
DHA	25.41	71.16	2.80
EPA+DHA	31.44	89.64	2.85

% (w/w) indicated the weight of fatty acid that calculated by using internal standard of C19:0 methyl ester

RESULTS

Characteristics of fish oil from by-product of tuna canning processing and fatty acid extract: The tuna fish oil from the by-product from tuna canning processing was a dark orange color and had specific tuna odor. The fatty acid composition of the oil was shown in Table 3 which was based on weight (% w/w). This fish oil had EPA and DHA levels of 6.0 and 25.4%, respectively. The fish oil of the by-product of canning processing met the quality of food grade fish oil therefore it was suitable for human consumption.

The fatty acids obtained from the saponification process are the intermediate product of urea crystallization. The initial process of urea crystallization was alcoholic KOH or NAOH hydrolysis of triglyceride structure to produce free fatty acids and glycerol (Wanasundara and Shahidi, 1999). In this process, the saponifiable matter was produced that further separated from fatty acids. The unsaponifiable fractions contained glycerol, sterol, and other non lipid components (Hodgson, 1995). In this process, the reduction of secondary oxidation level occurred but primary oxidation level increased.

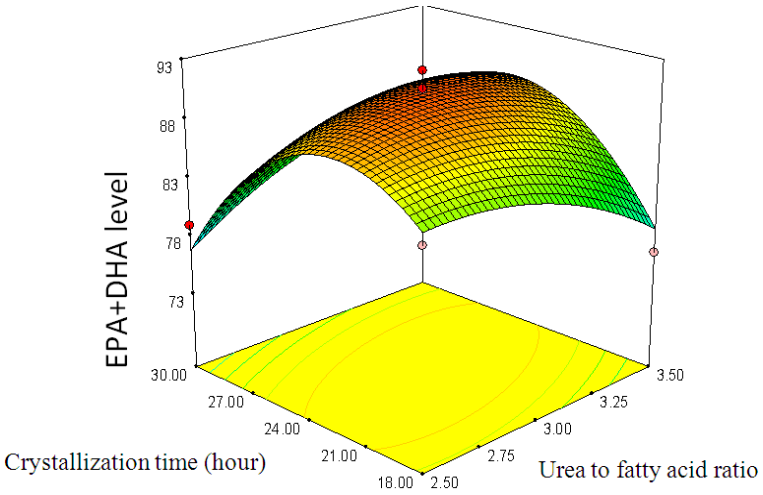
Optimization by response surface methodology: The fish oil from tuna canning processing had an EPA+DHA level of 31.4% (w/w). Response of EPA+DHA level at various urea to fatty acid ratio and crystallization time were 68.1-92.1% (Table 3) and showed a 2.17-2.93 enrichment

The response surface methodology was used to a define suitable model. The significant model was quadratic and cubic ($p<0.05$), whereas a linear model and their interaction (2 FI) was not significant ($p>0.05$). The model with highest order was cubic but this model was aliased, therefore the suggested model was quadratic. The second fitted model was based on lack of fit test and the model was fitted if $p>0.05$ ($p>5\%$), this indicated a significant lack of fit. Based on this test, a quadratic and a cubic models were suitable model ($p>5\%$). The next fitted model was based on model summary statistics. Among the fitted models, the suggested model was quadratic. Based on three fitted models, the suggested model was a quadratic. Analysis of variance showed that the quadratic model significantly affected the response (Table 4). The quadratic model had the least standard deviation compared to other models. Its adj. R^2 value was 0.89 and this meant that the urea to fatty acid ratio and the crystallization time affected the response by 89% and the remains were affected by other factors. The quadratic equation used to predict response at various urea to fatty acid ratio and crystallization time was: $Y = 24.440X_1 + 5.65X_2 - 8.71X_1^2 - 0.19X_2^2 + 1.17X_1X_2 - 12.95$. Negative sign of X_1^2 and X_2^2 showed that the response surface was maximum as shown in Fig. 1a. Maximum response was also identified by Liu *et al*. (2006) with used fish oil from enzymatic hydrolysis of tuna head.

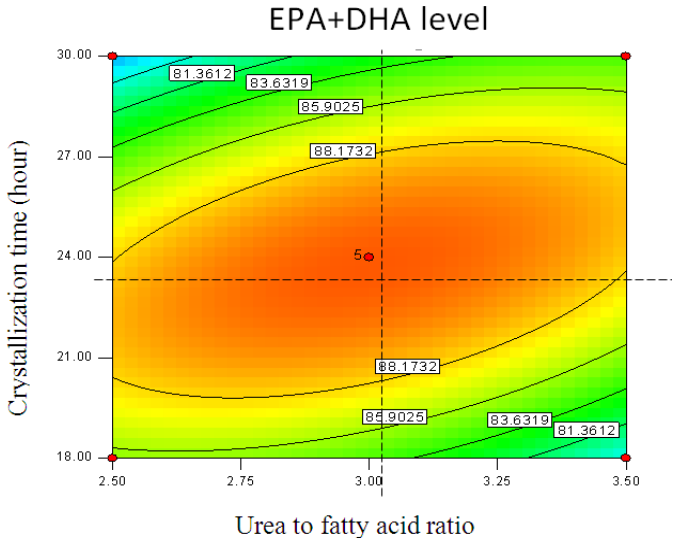
Verification of optimum condition was performed by preparing ω-3 concentrate at defined optimum condition which were urea to fatty acid ratio of 2.99:1 and crystallization time of 23.64 h. This was used to check the prediction accuracy obtained in the polynomial quadratic model. Urea crystallization process almost completely removed saturated fatty acids (C14:0; C16:0; C18:0) and completely removed C18:1 and C22:1 but did not remove C16:1 (Table 2). Urea could form crystal with saturated and monoenoic fatty acids (Hwang and Liang, 2001). Degree of ω-3 fatty acids enrichment of fish oil from by-product of tuna canning processing from verification (89.6%) was lower than the predicted response (90.4%). However, the difference was minimal therefore the quadratic

Table 4: Analysis of variance of the factors studied for the response surface model

Source	Sum of squares	df	Mean square	F value	p-value prob>F	
Model	404.24	5	80.85	21.020	0.0004	Significant
A-urea to fatty acid ratio	0.18	1	0.18	0.047	0.8343	
B-crystallization time	4.93	1	4.93	1.280	0.2948	
AB	49.42	1	49.42	12.850	0.0089	Significant
A^2	32.99	1	32.99	8.580	0.0221	Significant
B^2	338.32	1	338.32	87.960	<0.0001	Significant
Residual	26.93	7	3.85			
Lack of fit	22.34	3	7.45	6.500	0.0512	Not significant
Error	4.58	4	1.15			



(a)



(b)

Fig. 1: Response surface (a) and curve response (b) for the effect of urea to fatty acid ratio and crystallization time

polynomial model could be used to predict responses. A previous study (Liu *et al*., 2006) using tuna head oil produced by enzymatic hydrolysis, showed that ω-3 fatty acid level of predicted response was 89.38% and verified response of 85.02%.

Characteristics of ω-3 fatty acids concentrate from optimum condition: Characterization was performed on ω-3 fatty acid concentrate at optimum condition as shown in Table 2 except free fatty acids due to the fact that the chemical structure of concentrate was free fatty acids. The urea crystallization process reduced secondary oxidation level of oil (indicated by anisidine value) due to the saponification that dissolved secondary oxidation products in water. Oil color of concentrate was clearer and brighter that indicated by the lower Lovibond value. The Cu and Fe concentration of concentrate were higher than original fish oil. On the other hand, the phosphoric concentration was lower in concentrate than in original fish oil.

DISCUSSION

Tuna oil is a particularly good source of DHA. EPA and DHA levels of tuna (*Thunnus thynnus*) oil is 4.7 and 36.3%, respectively (Visentainer *et al*., 2007). Whereas, Howe *et al.* (2002) reported that tuna oil from by-product of meal processing had EPA level of 4.8%

and DHA level of 22.4%. The raw material of fish oil from the by-product of canning processing was tuna fillet, without viscera, red muscle and head. Compared to fish oil from by product of meal processing, that fish oil had raw material as solid waste of canning processing which consisted of viscera, head including eyes, fins, and red muscle. Tuna eyes are the source of ω-3 fatty acids, particularly DHA. Fish oil extracted by solvent from tuna eyes had EPA level of 5.1% and DHA level of 26.2% for Yellowfin and EPA level of 5.9% and DHA level of 24.1% for Skipjack (Yuwono, 1993). Fish oil in this study was obtained from *Thunnus* species, due to the fact that during canning processing, various tuna were mixed together. The most abundant type of tuna used in canning processing was Yellowfin and Skipjack (Elizabeth, 1992). EPA and DHA levels of Yellowfin were 5.0 and 22.0%, respectively (Kinsella, 1986). Various factors affect the fatty acid composition of fish oil. The quality of fish food such as phytoplankton influences ω-3 fatty acids of fish oil. Furthermore, factors affecting fatty acid composition of fish oil are species, sex, sexual maturity, body size, environment where the fish were caught, water temperature, type of food the fish are feeding on, and seasons. It is reported that these factors result in changes in tuna oil composition (Visentainer *et al*., 2007). It was thought that secondary oxidation products partitioned to the unsaponifiable matter or dissolved in water from alkali solution. Whereas primary oxidation products such as peroxides were not able to dissolve in water and accumulated in oil, therefore there was an increase in peroxide value.

Results from a previous study (Elizabeth, 1992) showed the highest EPA+DHA level of concentrate from by-product of tuna canning processing was 46.07% with EPA+DHA level in original oil of 10.8% and degree of enrichment of 4.27 times. The higher degree of enrichment may be due to the lower level of ω-3 fatty acids therefore it is possible that other fatty acids rather than ω-3 could form crystal with urea.

Analysis of variance (Table 4) showed that urea to fatty acid ratio, crystallization time and both interactions affected the response significantly. Their effect was shown in Fig. 1 where the response increased as urea to fatty acid ratio increased to the highest response of between 2.75 and 3.00. The further increase in urea to fatty acid ratio decresed the response. Low urea to fatty acid ratio (limited amount of urea and high amount of fatty acid) produced low EPA and DHA level. Urea inclusion compound formation occured because urea formed hexagonal crystal that consisted of 6 urea molecules (Hayes, 2002). At limited amount of urea molecule, there was a limitation to form hexagonal structure for entrapping guest compound. Therefore, at low urea to fatty acid ratio, some fatty acids could not form inclusion complexes with urea that made for lower level of EPA+DHA.

There is a preference of fatty acids to form urea inclusion complexes (Liu *et al*., 2006). Total elimination of all saturated fatty acids was impossible due to the fact that some of saturated fatty acids could not form a complex with urea during crystallization. It was proposed that the decrease in urea complex formation was caused by an increase in unsaturation and a decrease in the chain length (Guil-Guerrero and Belarbi, 2001). Therefore, long chain saturated fatty acids were prefered to form complex with urea over short chain saturated fatty acids. Highly unsaturated fatty acids tends not to form urea inclusion complexes. At excessive amount of urea and limited amount of fatty acids (high urea to fatty acid ratio), it was thought that there was intensive inclusion complex formation. The abundance of urea caused high availability of hexagonal urea stuctures in methanol that could form complex with guest compounds. The likelihood of fatty acids to form urea was also high. It was possible that some of ω-3 fatty acids formed complexes with urea that produced a low level of ω-3 fatty acids in concentrate. According to Liu *et al.* (2006), EPA had higher preference to form an urea inclusion complex than DHA. Hwang and Liang (2001) reported that EPA was found at urea complexing fraction, indicated that at certain condition, such as high availability of urea, EPA could form a complex with urea.

The crystallization time significantly affected the response, however it was not thought to be caused by dynamic conditions during crystallization. Urea complex formation is reversible. The formed urea complex can decompose into hexagonal urea and fatty acids, however, factors affecting the dynamic condition i.e., temperature (Engkvist *et al*., 2000) was fixed in this study. The tuna oil had a high degree of oxidation (Table 2). Oxidation products in oil were the initiator of autooxidation, therefore ω-3 fatty acids could be oxidized. Although urea crystal could protect oil from oxidation (Stout *et al*., 1990), but protective effect was found for fatty acids that formed complex with urea. Therefore, after definite crystallization time, EPA+DHA level of concentrate decreased. ω-3 fatty acids were very succesptible to oxidation although at storage temperature of 4°C (Cho *et al*., 1987). In this study, the crystallization temperature was 10°C that it was possible for ω-3 fatty acids to oxidize. Peroxide by chain reactions initiated oxidation reaction and it was supposed that after definite crystallization time, ω-3 fatty acid were oxidized that reduced their level.

The stationary point could be predicted from the response curve (Fig. 1b). The real value of the stationary point was obtained by canonic analysis which were the urea to fatty acid ratio of 2.99:1 and the crystallization time of 23.64 h. Predicted response at optimum condition was the EPA+DHA level of 90.4% (w/w). The tuna oil had a higher level of DHA than

EPA, therefore urea complex formation was supposed to be low due to the different preference of urea to form complex with EPA and DHA. Urea had preference to form complex with EPA and urea complexing fraction was supposed to contain EPA rather than DHA that produce more DHA in concentrate.

Most of secondary oxidation products are polar compounds and able to dissolve in water. Otherwise, primary oxidation products indicated by peroxide value increased due to the fact that peroxide tended to dissolve in oil rather than water. It was thought that during crystallization, oxidation process occurred slowly and produced more peroxides. A similar phenomenon was reported (Liu *et al*., 2006) that during ω-3 fatty acids concentrate preparation, the peroxide value increased due to oil susceptibility to oxidation.

The improvement in oil color was caused by decreasing heme concentration of the oil during concentrate preparation particularly at pH adjustment after the saponification. Heme is a pigment binding protein. After the saponification, fatty acids were extracted and added by HCl to convert sodium fatty acids into free fatty acids. During this process, the pH value was 1 and protein coagulation (including heme) occurred. Also during this process, other pigment of fish oil was supposed to be unsaponified and removed. The reason of the increase in Fe and Cu concentrations was still unknown. Actually, all water soluble matters would be dissolved in water from alkali solution during saponification. Water added during the saponification could dissolve phosphatides. According to Hodgson (1995), phosphoric compounds in oil are in the form of phosphatides that exhibit gum property and can be removed by the saponification process. In general, the quality of ω-3 fatty acids concentrate fulfilled the food grade fish oil standard according to IFOMA except for Cu and Fe concentration.

CONCLUSION

Fish oil by-product of tuna canning processing was suitable as a food and for human consumption. This oil is a cheap source of ω-3 fatty acids for food, pharmaceutical and nutrition use. The DHA level was higher than EPA in this oil and suitable as a source of DHA rather than EPA. The urea crystallization was affected by the urea to fatty acid ratio and crystallization time. The optimum conditions for producing ω-3 fatty acids concentrate were the urea to fatty acids ratio of 2.99:1 and the crystallization time of 23.64 h, whereas predicted and verified response of 90.4 and 89.6%, respectively. Generated polynomial quadratic equation developed in this study could be used to predict response. The ω-3 fatty acids concentrate had lower oxidation levels, P concentration, viscosity and density, as well as better color than original oil. However, Fe and Cu concentration increased during this process. The changes were due to the saponificaton processing as one step in concentrate preparation, however there were no reasonable reasons for the increase of Fe and Cu concentration. The oil from the by-product of fish processing could be processed to ω-3 fatty acids concentrate without any refining processes.

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