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Research Article

Determine the Factors that Affect the Enrichment Process of High Bioactive Substance from Pangasius Oil

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Abstract: The present study aims to isolate the fatty acid from pangasius fish oil by hydrolysis method and determine the factors that affect the enrichment of the high bioactive chemical elements such as Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) which are two polyunsaturated fatty acids in Omega-3 group by urea complexation process. At the same time, the optimization determined the relationship between mathematics and technology to choose the best technological solution. The recovery efficiency of fish oil reached maximum value at the urea-to-fatty acid ratio of 3.2, complexation time of 16 hours and complexation temperature of -10.6°C. Under the optimal conditions, the enrichment ratio of DHA and EPA also reached maximum value of 75.5 and 78.9% in order to enhance the value of pangasius oil raw material and significantly improve the quality of final product.

Keywords: Enrichment, fish oil, optimization, pangasius, urea complexation

INTRODUCTION

Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) are two polyunsaturated fatty acids in Omega-3 group. These acids are major components of brain and retina which prevented the eyes diseases and the memory impairment in the old (Can *et al*., 2002; Shahidi and Wanasundara, 1998). Besides, DHA is essential for the development of brain and retina in children, especially for third trimester foetus and infant (Shahidi and Wanasundara, 1998; Eduardo, 2010). In addition, these fatty acids reduce cholesterol and triglyceride in blood which prevent cardiovascular diseases (Simopoulos, 1997). The demand for food such as fish oil capsules, high level content of DHA milk, supplementary the fatty acids (DHA, EPA) food oil was increasing while the raw materials to enrich these fatty acids were deficient (Can *et al*., 2002; Shahidi and Wanasundara, 1998). Most research in over the world mainly concentrated on determining the content of DHA, EPA in food or detecting DHA, EPA in some sea-fish, microalgae and creating high DHA product from algae. There were some research about the property, role and function of DHA in children's brain development and in the metabolism of cholesterol into derivatives that did not cause the obstruction to blood vessels, decrease heartbeat and myocardial infarction (heart attack) (Simopoulos, 1997; Eduardo, 2010). However, the study of the purification and enrichment of DHA, EPA on freshwater fish was not considered, especially from Vietnam Pangasius fish oil.

Pangasius fish (*Pangasius hypophthalmus*) is one of the most grown fish in Mekong Delta area of Vietnam and also has high export value. The annual production of pangasius fish reached 1.28 million tons (Luc, 2012) that primarily exported the frozen fish fillet which take 30% of fish weight. The large amount of waste was head, bones, skins and fat while fish oil take over 15, 3% of fish weight. There was over 200 thousand tons of pangasius fish oil (Luc *et al*., 2013) that were not enhanced the value and utilized effectively every year. In this study, the fish oil was used as a supplemental source of fatty acids that contained bioactive substances such as DHA and EPA to put into processing of functional food. Based on that demands, the determination of factors that affect the enrichment of high bioactive (omega-3) components in fish oil was essential.

The enrichment of DHA and EPA was a combination of different techniques: hydrolysis method to isolate the fatty acid from pangasius fish oil and urea complexation method at low temperature to enrich the omega-3 unsaturated fatty acids. At the same time, the optimization determined the relationship between mathematics and technology to achieve the maximum value of the efficiency of high bioactive substances' enrichment from fish oil.

MATERIALS AND RESEARCH METHODS

Pangasius fish oil raw material were obtained from Thuan An Limited Company at An Giang Province and

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brought to the laboratory of Department of Food Technology, University of Technical Education HoChiMinh City for the study.

Hydrolysis method: Can *et al.* (2002) and Shimada *et al*. (1997) Hydrolysis of oil in alkaline environment or has known as fish oil transester reaction by using the heat stirring Labinco model L-80 equipment from Netherlands. The weight of experimental pangasius oil was 20 grams, the catalyst KOH was mixed with 95% ethanol in magnetic stirrer, room temperature, time of 5 to 30 min, heating of 60°C, reaction time of 60 min, KOH-to-fish oil ratio of 2/1.

Urea complexation method: Wanasundara and Shahidi (1999). The free fatty acids were obtained from pangasius fish oil after hydrolysis. They were mixed with urea (10%, w/v) in 95% aqueous ethanol and heated at 60-70°C with stirring until the whole mixture turned into a clear homogeneous solution. The ratio of urea-to-fish oil was certain and then frozen this solution at -5°C, -10°C and -15°C in thirty hours to remove crystals by filtration. The filtrated water was diluted with an equal volumn of distilled water and acidified to pH = 1-2 with 2M HCl. An equal volumn of hexane or petroleum ether was added and stirred well, then tranferred to separatory funnel. The top hexane layer containing liberated fatty acids was separated from the bottom aqueous layer containing urea and dried over anhydrous. The solvent was subsequently gentle removed by a rotary evaporator to recover the free fatty acids containing mainly unsaturated fatty acids.

Optimization method: In this study, the mathematical model was built by Box-Hunter (Canh, 2004), with k = 3, n_0 = 4 about the relations between y and x_1, x_2, x_3 (Wanasundara and Shahidi, 1999; Luc *et al*., 2013) according to the equation below:

$$y = b_0 + \sum_{1\le j\le k} b_j x_j + \sum_{1\le j\le k} b_{ji} x_j x_1 + \sum_{1\le j\le k} b_{jj} x^2_j \quad (1)$$

where,
y = Output
x_i = Input
b_0, b_j, $b_{j\,i}$ *và* b_{ii} = The regression coefficients

The objective function y was the efficiency of recovering oil containing unsaturated fatty acid after the urea complexation. Factors related to objective function were urea-to-fatty acid ratio (x_1), complexation time (x_2, h), complexation temperature (x_3, °C). Other factors were constant.

The calculation of result: + Determined fatty acid by gas chromatography equipment Shimadzu G-17 using semi-quantitative method of Methyl Ester metabolism.
+ Hydrolysis performance:

$$H = \frac{m_c}{m_d} \times 100\% \quad (2)$$

where,
m_c = Fish oil weight after hydrolysis
m_d = Initial fish oil weight

$$+\ \text{DHA enrichment ratio (\%)} = \frac{m_c \times DHA_{sp}}{m_d \times DHA_{nl}} \quad (3)$$

where,
m_c = Fish oil weight after removing the crystals
m_d = Initial fish oil weight
DHA_{sp} = Content of DHA in product
DHA_{nl} = Content of DHA in raw material

$$+\text{EPA enrichment ratio (\%)} = \frac{m_c \times EPA_{sp}}{m_d \times EPA_{nl}} \quad (4)$$

where,
m_c = Fish oil weight after removing the crystals
m_d = Initial fish oil weight
EPA_{sp} = Content of EPA in product
EPA_{nl} = Content of EPA in raw material

Data processing method: Using mathematical tools and Microsoft Excel 2010 software and Matlab 7.1 programming for data processing, optimizing the urea complexation and calculate results.

RESULT AND DISCUSSION

Determine the components of fatty acids from pangasius fish oil: The components of fatty acids from pangasius fish oil in Table 1.

Pangasius fish oil contained high amount of unsaturated fatty acids such as 38.9% oleic acid, 13.9% linoleic acid, 0.66% α-linolenic acid, 0.31% arachidonic acid, 0.45% Eicosapentaenoic Acid (EPA) and 0.5% Docosahexaenoic Acid (DHA). Thus, pangasius fish oils have been recognized as good sources of Polyunsaturated Fatty Acids (PUFA) that are widely used for pharmaceutical purposes and food supplements.

Determine the factors that affect the hydrolysis:

Effect of KOH concentration: Determine the concentration of KOH so that the hydrolysis reaction occurred completely and the most efficiently. We started to examine the hydrolysis reaction with the concentration of KOH at 0.5N, 1N, 1.5N, 2N in 1 h with the temperature of 60°C, ethanol concentration of 95% and the ratio KOH-to-fish oil of 2:1. The result determining the effect of KOH concentration was presented in Fig. 1.

The above results showed that the efficiency of reaction depended on the concentration of KOH. If the concentration increases, the efficiency will increase until reaching 95.01% at 1N KOH and then reduces gradually. Therefore, we chose the concentration of KOH at 1N to make the reaction occur completely, the

Table 1: The components of fatty acids from pangasius oil

TT	Shortened formula	Fatty acid components	Content (g/100 g total fatty acid)
1	C12:0	Lauric acid	0.19
2	C14:0	Myristic acid	5.29
3	C16:0	Palmitic acid	31.55
4	C16:1	Palmioleic acid	0.70
5	C18:0	Stearic acid	7.62
6	C18:1n-9	Oleic acid	38.9
7	C18:2n-6	Linoleic acid	13.9
8	C18:3n-3	α-linolenic acid	0.66
9	C20:4n-6	Arachidonic acid	0.31
10	C20:5n-3	Eicosapentaenoic acid	0.45
11	C22:6n-3	Docosahexaenoic acid	0.50

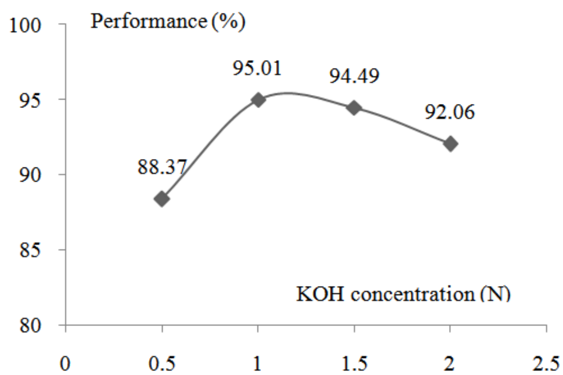

Fig. 1: Effect of KOH concentration to efficiency

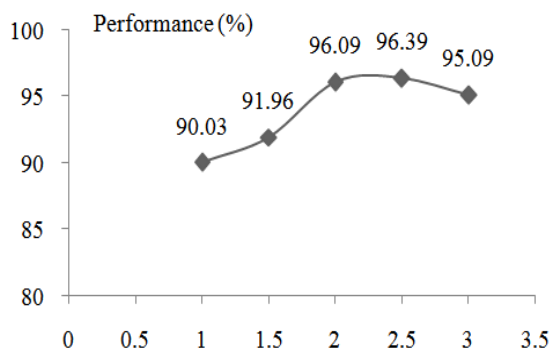

Fig. 2: Effect of KOH-to-fish oil ratio to efficiency

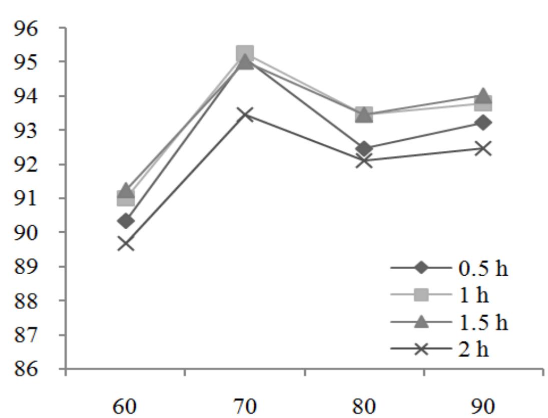

Fig. 3: Effect of time and temperature to efficiency

most efficiently and reduce KOH after reaction to minimum.

Effect of KOH-to-fish oil ratio: Determine KOH-to-fish oil ratio so that the hydrolysis reaction occurred completely and the most efficiently, we conducted experiment with the KOH-to-fish oil ratio at 1/1, 1.5/1, 2/1, 2.5/1, 3/1 in 1 h with the temperature of 60°C, 1N KOH concentration and 95% ethanol concentration. The result determining the effect of KOH-to-fish oil ratio was presented in Fig. 2.

The above results showed that the efficiency of reaction depended on the KOH-to-fish oil ratio. If the ratio of KOH to fish oil were 2.5, the efficiency would reach highest value of 96.39%. Then, the higher ratio of KOH-to-fish oil was, the more amount of KOH after reaction created and pH increased gradually. Therefore, we need to use more HCl to neutralize the solution but do not significantly alter the efficiency. Finally, KOH-to-fish oil ratio should be 2.5 to make the hydrolysis reaction occur completely and the most efficiently and reduce KOH after reaction to minimum.

Effect of time and temperature: Determine the time and temperature so that the hydrolysis reaction occurred completely and the most efficiently, we conducted experiment at the temperature of 60°C, 70°C, 80°C, 90°C in 0.5 h, 1 h, 1.5 h, 2 h, respectively, 1N KOH concentration, 95% ethanol concentration and the ratio of KOH-to-fish oil of 2.5. The result determining the effect of time and temperature was presented in Fig. 3.

The results showed that if the reaction occurred longer and at higher temperature, the oil would have more time to expose with oxygen then create the peroxide. It would reduce the concentration of unsaturated fatty acids in the oil, so that the hydrolysis efficiency did not increase significantly. Therefore, we chose time of 1 h and temperature of 70°C to make the hydrolysis reaction occur completely and the most efficiently.

Through some above hydrolysis experiments, we selected the appropriate experimental conditions for 3 samples at 1N KOH, 95% ethanol concentration, the KOH-to-fish oil ratio of 2.5, temperature of 70°C and time of 1 h. Under these conditions, the efficiency of reaction was 94.9% that presented in Table 2.

The hydrolysis reaction that obtained fatty acids from fish oil had high efficiency. The oil after hydrolysis reaction was pale yellow, transparent and had a feature odour of fish oil.

Determine the factors that affect urea complexation reaction:

Effect of urea-to-fatty acid ratio: Starting to examine the hydrolysis reaction at urea-to-fatty acid ratio of 2:1, 2.5:1, 3:1, 3.5:1,4:1, respectively, at 10°C, 24 h. The result was determined by a diagram as per Fig. 4.

Table 2: Hydrolysis efficiency of fish oil

TT	m_1 (g)	m_2 (g)	H (%)	H_{tb} (%)
1	20.03	19.02	94.95	
2	20.09	19.04	94.77	94.9
3	20.01	19.01	95.0	

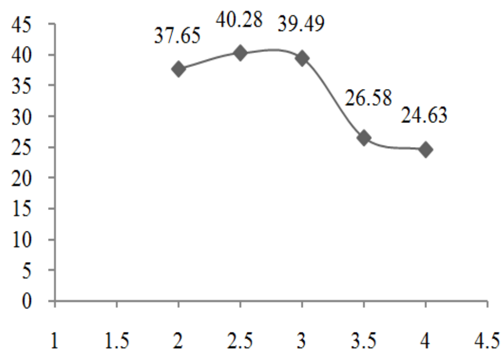

Fig. 4: Effect of urea-to-fatty acid ratio to efficiency

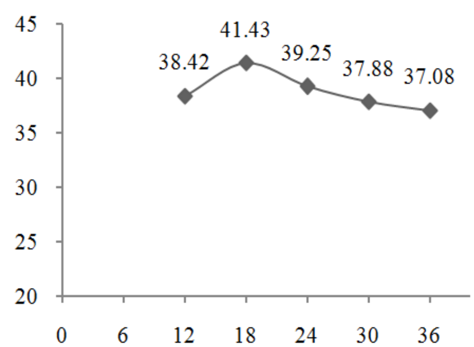

Fig. 5: Effect of time to efficiency

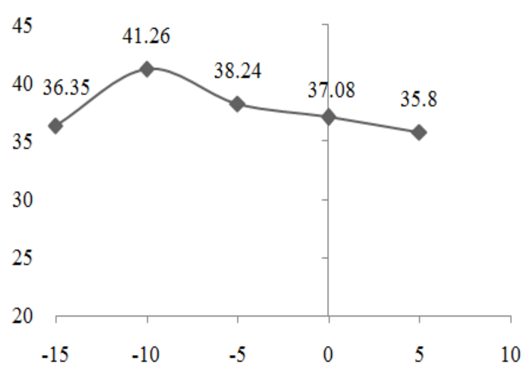

Fig. 6: Effect of temperature to efficiency

Statistics analysis showed that urea-to-fatty acid ratio significantly affected recovery efficiency of urea complexation with 95% reliability. The difference between the urea-to-fatty acid ratio and recovery efficiency was statistically significant. The more urea added, the more complexes of saturated fatty acid and urea were formed in fish oil sample because urea covered the linear saturated fatty acid and unsaturated fatty acid with one double bond in its hexagonal crystal structure and left the polyunsaturated fatty acid because these one have large molecular weight and big space structure. However, in spite of increasing the urea-to-fatty acid ratio, some saturated fatty acid were not formed to urea complexation, hence the saturated fatty acid was removed completely from solution and make the recovery efficiency of unsaturated fatty acid after the complexation process low. Therefore, we chose a urea-to-fatty acid ratio of 2.5 to get the highest efficiency.

Effect of time: Start to examine the complexation reaction at time of 12 h, 18 h, 24 h, 30 h, 36 h with urea-to-fatty acid ratio of 2.5 and the temperature of -10°C. The result was shown in Fig. 5.

The results showed that the longer time was, the more complexes between saturated fatty acid and urea were formed and get the recovery efficiency of 41.43% in 18 h. If we continued to increase the complexation time, the purity of unsaturated fatty acid would reduce and the content of DHA in product also would be reduced as well because DHA was degraded more easily than other fatty acids. Therefore, 18 h was the best time for the urea complexation to get the highest performance.

Effect of temperature: Examine the urea complexation at temperature of -15°C, -10°C, -5°C, 0°C, 5°C with urea-to-fatty acid ratio of 2.5 and complexation time of 18 h. The result was presented in the graph Fig. 6.

According to Fig. 6, the urea complexation was exothermic reaction. The lower temperature was, the faster complexes were formed. If the temperature reduced from 5°C to -10°C, the urea complexes would be increased and get the efficiency of 41.26% at -10°C. However, if the temperature was too low, the solvent and complex would be solidified, thereby the complication performance and the content of DHA, EPA in the product would be reduced. The performance reached maximum value of -10°C, so we chose -10°C as a complexation temperature of reaction.

Building the mathematical model to describe the enrichment of DHA and EPA in fish oil: On the basis of the system analysis and approach, it can be seen that the oil recovery process after urea complexation containing unsaturated fatty acid mainly depended on the parameters such as: a urea-to-fatty acid ratio (x_1), complexation time (x_2, h); complexation temperature (x_3, °C) that affect objective function. Therefore, the technological mode for the recovering of fish oi needed to be determined in order to obtain highest unsaturated fatty acids which meant objective function had to reach maximum level. As the consequences, in this study, the mathematical model was built by Box-Hunter (Canh, 2004) with $k = 3$, $n_0 = 4$ about the relations between y with x_1, x_2, x_3.

Table 3: Level of technological parameters in the experimental design

	Levels					
Parameters	- α (-1, 414)	Low -1	Central 0	High +1	+ α (1, 414)	Deviation ΔZ_i
x_1 (%)	1.793	2	2.5	3.0	3.207	0.5
x_2 (h)	15.516	18	24	30	32.484	6
x_3 (°C)	-17.07	-15	-10	-5	-2.93	5

Table 4: Matrix of Box-Hunter's secondary orthogonal experimental method with k = 3, n_0 = 4

N	x_0	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	x_1^2-2/3	x_2^2-2/3	x_3^2-2/3	y
1	1	1	1	1	1	1	1	0.33	0.33	0.33	39.1
2	1	1	1	-1	1	-1	-1	0.33	0.33	0.33	37.29
3	1	1	-1	1	-1	1	-1	0.33	0.33	0.33	42.56
4	1	-1	1	1	-1	-1	1	0.33	0.33	0.33	39.5
5	1	-1	-1	-1	1	1	1	0.33	0.33	0.33	38.29
6	1	-1	-1	1	1	-1	-1	0.33	0.33	0.33	35.1
7	1	-1	1	-1	-1	1	-1	0.33	0.33	0.33	37.74
8	1	1	-1	-1	-1	-1	1	0.33	0.33	0.33	42.9
9	1	1.414	0	0	0	0	0	1.33	-0.67	-0.67	40.55
10	1	-1.414	0	0	0	0	0	1.33	-0.67	-0.67	42.26
11	1	0	1.414	0	0	0	0	-0.67	1.33	-0.67	38.05
12	1	0	-1.414	0	0	0	0	-0.67	1.33	-0.67	41.33
13	1	0	0	1.414	0	0	0	-0.67	-0.67	1.33	41.87
14	1	0	0	-1.414	0	0	0	-0.67	-0.67	1.33	35.65
15	1	0	0	0	0	0	0	-0.67	-0.67	-0.67	42.05
16	1	0	0	0	0	0	0	-0.67	-0.67	-0.67	40.3
17	1	0	0	0	0	0	0	-0.67	-0.67	-0.67	41.8
18	1	0	0	0	0	0	0	-0.67	-0.67	-0.67	41.5

Table 5: Recovery efficiency of fatty acid

	m_1 (g)	m_2 (g)	H (%)	H_{tb} (%)
1	10	4.43	44.3	
2	10.02	4.45	44.41	44.42
3	10.01	4.46	44.55	

Number of experiment in this method was determined as the follow equation:

$$N = 2^k + 2.k + n_0 = 2^3 + 2x3 + 4 = 18 \quad (5)$$

In order to the experimental matrix is orthogonal, α = 1, 414

x_j is variables of objective function:

$$j = 1 \div 3 \quad (6)$$

where,

x_1 = Urea-to-fatty acid ratio needed for urea complexation from 2 to 3

x_2 = Complexation time from 18 to 30 h

x_3 = Complexation temperature from - 5°C to -15°C

With limited domain is:

$$\Omega_X = (-1,414 \leq x_1, x_2, x_3 \leq 1, 414) \quad (7)$$

From the conditions of technology, experimental process as well as production process, the range of technological parameters used in this research was presented in the Table 3.

The experiment was carried out with the level of technological parameters in Table 3 and experimental planning matrix (Box-Hunter). The results of objectives at different levels were presented in Table 4.

The results in Table 4 was calculated by using Microsoft Excel 2010 to determine coefficients of the experimental models (the regression equation). Subsequently, these coefficients were checked about the significance by Student standard and about the compatible of the regression equation by Fisher standard (Canh, 2004; Luc and Hai, 2008; Wanasundara and Shahidi, 1999). The regression equation of recovery efficiency of urea complexation with fatty acid was presented bellow:

$$y = 41.81+0.734x_1-0.821x_2+0.736x_3-1.615 x_1x_2+0.887x_2x_3-1.211x_2^2-1.675x_3^2 \quad (8)$$

The results of the optimization determined the relationship between mathematics and technology by the recovery efficiency of fish oil, urea-to-fatty acid ratio, complexation time and complexation temperature.

Using Excel-Solver to find the values of the regression Eq. (9) for y_{max} at $x_1^{opt} = 1.414$; $x_2^{opt} = -1.33$; $x_3^{opt} = -0.133$ and then $y_{max} = 44.858$. Switching to real variables, the complexation urea-to-fatty acid ratio was determined of 3.207 at complexation time of 16 h and complexation temperature of -10.6°C.

Conducted 3 replicated experiments under optimal conditions (the urea-to-fatty acid ratio of 3.2, complexation time of 16 h and complexation temperature of -10.6°C) to obtain the result that presented in Table 5.

The concentration of DHA and EPA in the final product was evaluated as: DHA = 0.85% and EPA = 0.8%, hence, we determined the enrichment rate of DHA and EPA:

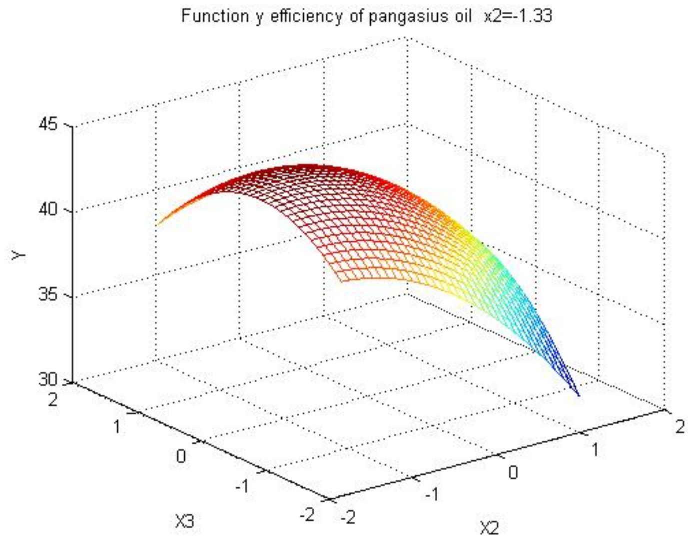

Fig. 7: Function y efficiency of pangasius oil

$$H_{DHA} = \frac{4.44 \times 0.85}{10 \times 0.5} = 75.5(\%) \quad (9)$$

$$H_{EPA} = \frac{4.44 \times 0.8}{10 \times 0.45} = 78.9\ (\%) \quad (10)$$

The above results showed that optimizing the recovery process of urea complexation under these conditions: urea-to-fatty acid ratio of 3.2, time of 16 h and temperature of -10.6°C, the efficiency will reach the maximum value of 44.42% and the enriching rate of DHA and EPA (H_{DHA}, H_{EPA}) were 75.5% and 78.9%, respectively.

The simulation of the mathematical models of the objective functions (9), in 3D were performed in Fig. 7.

The results showed that quality Pangasius fish oil did not significantly change after 2 months preservation.

CONCLUSION

Determined the components and the content of fatty acids in pangasius oil which were oleic acid (38.9%), linoleic acid (13.9%), docosahexaenoic acid (0.5%) and eicosapentaenoic acid (0.45%). Determined the factors which affected a hydrolysis process were 1N KOH catalyst, solvent ethanol concentration of 95%, the KOH-to-fish oil ratio of 2.5, temperature of 70°C and time of 1 hour. Under these conditions, the hydrolysis efficiency reached to 94.9%.

Optimization of urea complexation reaction showed the relationship between mathematics and technology to achieve the highest recovery efficiency and increase the enrichment of DHA and EPA in product. Under optimal conditions which were the urea-to-fatty acid ratio of 3.2, complexation time of 16 h and complexation temperature of -10.6°C, the recovery efficiency reached maximum level of 44.42% and the content DHA and EPA enrichment of 75.5, 78.9%, respectively.

The above results concluded that fish oil contained high Polyunsaturated Fatty Acid (PUFA) to widely using for pharmaceutical purpose and supplemental food. By the optimization method, DHA and EPA enrichment process was the background for choosing the technological mode in order to enhance the resource of pangasius oil.

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