

Research Article

Multiobjective Optimization of the Enrichment Process DHA and EPA From Pangasius Fish Oil

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Abstract: Fish oils have been recognized as good sources of Polyunsaturated Fatty Acids (PUFA), which are widely used for pharmaceutical purpose and food supplement. In this study, the multi-objective optimization method was used to describe the enrichment process of DHA, EPA, omega-3 from pangasius fish oil with the urea-to-fish oil ratio, complexation temperature and urea complexation time which affected the enrichment's level of polyunsaturated fatty acid. The multi-objective optimization result has determined the technological mode of enrichment process at the urea-to-fish oil ratio of 2/1, complexation temperature of -11°C and urea complexation time of 18.6 h. Under these conditions, the enrichment efficiency of DHA, EPA and omega-3 reached to maximum level of 120.06, 58.4 and 179.5% and it was the basis to establish the technology mode for the enrichment process from pangasius fish oil. The result was totally compatible with experiment and able to apply in industrial production in order to create the fish oil which contented high polyunsaturated fatty acid and better in quality.

Keywords: Enrichment, fish oil, multi-objective optimization, pangasius, urea complexation

INTRODUCTION

Pangasius fish (*Pangasius hypophthalmus*) is the freshwater fish which commonly grown in Mekong Delta area of Vietnam. The annual production of pangasius fish reached 1.28 million tons (Ministry of Agriculture and Rural Development, Vietnam 2010), 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 fat take over 15.3% of fish weight (Luc *et al.*, 2013). The components of pangasius fish fat contained bioactive substances such as DHA and EPA that was supplement of Polyunsaturated Fatty Acid (PUFA) and was used as pharmacia and supplemental source and pharmaceutical aim (Can *et al.*, 2002; Shahidi and Wanasundara, 1998).

Docosahexaenoic acid (DHA, 22:6(n-3)) and eicosapentaenoic acid (EPA, 20:5(n-3)) are two unsaturated 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 (Shahidi and Wanasundara, 1998; Eduardo, 2010). Besides, Docosahexaenoic acid (DHA) is essential for the development of the brain, healing the burns and retina children, especially in supplying nutrition for third trimester foetus and infant (Shahidi and Wanasundara, 1998; Eduardo, 2010). In additional, these fatty acids reduce cholesterol and triglycerides in

blood that prevent cardiovascular disease and reduce the risk of stroke by heart disease (Simopoulos, 1997).

Nowadays, there are many researches of the effect of Omega-3, especially DHA to human's health. The researches about the factors containing omega-3 from oil of ocean fish such as salmon, mackerel, tuna and other types of marine microalgae are widely used in the producing the fish oil capsules, high content of DHA milk, supplemental fatty acids (DHA, EPA) food oil... However, the study of the extraction and enrichment of DHA, EPA from pangasius fish oil was not considered, hence, the supplemental source of pangasius fish oil was not used effectively. Moreover, the consume demands of high content of DHA and EPA products increases while the supplemental source of enriching these fatty acids are shorted. Based on that demands, the research of multi objective optimization of the enrichment process of DHA and EPA from pangasius fish oil is urgent and essential.

In this study, using optimization problems with multi-objective approached by methods of utopian point (Canh, 2004; Luc and Hai, 2008) to establish the technology mode of enriching the substance of birth primarily DHA, EPA and omega-3 from fish oil. Thereby improving the quality in refining fish oil, the optimization problems confirmed a close relationship between technology and mathematics. Shedding light on the technology under systematic approach is a modern approach by mathematical thinking and is the

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basis to set the technology mode for processing the value-added products.

MATERIALS AND RESEARCH METHODS

Pangasius fish oil raw material were obtained from Thuan An Limited Company at An Giang Province and brought to the laboratory of Department of Food Technology, University of Technical Education HoChiMinh City for the research.

Using experimental research methods, combining with the biochemical research, the support from mathematical tools and the optimization algorithms to detect new properties and the relationship between parameters, supported by Microsoft Excel software, Matlab 7.1 programming and confirmed by experiments.

In this study, the mathematical model was built by Box-Hunter's secondary orthogonal experiment method (Canh, 2004), with $k = 3$, $n_0 = 6$ about the relations between y and x_1 , x_2 , according to the equation below:

$$y = b_0 + \sum_{1 \leq j \leq k} b_j x_j + \sum_{1 \leq j < i \leq k} b_{ji} x_j x_i + \sum_{1 \leq j \leq k} b_{jj} x_j^2 \quad (1)$$

where,

y = Output

x_i = Input

b_0, b_j, b_{ji} và b_{jj} = The regression coefficients

In this report, there are three objectives: recovery efficiency of DHA (y_1); recovery efficiency of EPA (y_2); recovery efficiency of Omega-3 (y_3). These factors related to the objectives: the ratio of urea-to-fatty acids (x_1), complexation time (x_2) and complexation temperature (x_3). The others factors were considered as unchanged during the research.

Using the urea complexation method (Wanasundara and Shahidi, 1999): This is the method by using urea to create the complexation with saturated fatty acid at low temperature and eliminate these acids from the first fatty acid mixture.

The free fatty acids were obtained from pangasius fish oil after hydrolysis. The hydrolysis conditions were 1N KOH; the ratio of KOH-to-fish oil of 2.5, temperature of 70°C, time of 1 hour and the hydrolysis efficiency reached to 94.9% (Luc and Minh, 2014). The free fatty acid then were mixed with urea (10%, w/v) in 95% 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 from 2 to 3 according to mol and then frozen this solution from -15 to -5°C in 18 to 30 h, after that the crystals were removed by filtration (Shimada *et al.*, 1997). 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 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.

Determined the compositions of fatty acid DHA, EPA and omega-3 ($C_{18:3}$; $C_{20:5}$; $C_{22:6}$) by gas chromatography equipment Shimadzu G-17 using semi-quantitative method of Methyl Ester metabolism.

The calculation of result:

$$+ \text{DHA enrichment ratio (\%)} = \frac{m_c \times DHA_{sp}}{m_d \times DHA_{nl}} \quad (2)$$

In which,

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:

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

In which,

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:

$$+ \text{Omega-3 enrichment ratio (\%)} = \frac{m_c \times \omega_{sp}}{m_d \times \omega_{nl}} \quad (4)$$

In which,

m_c : Fish oil weight after removing the crystals

m_d : Initial fish oil weight

ω_{sp} : Content of omega-3 in product

ω_{nl} : Content of omega-3 in raw material.

RESULTS AND DISCUSSION

Building the experimental design: On the basis of the system analysis and approach, it can be seen that the enrichment process of DHA, EPA and omega-3 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 functions: a recovery efficiency of DHA (y_1), a recovery efficiency of EPA (y_2), a recovery efficiency of omega-3 (y_3). In this study, the mathematical model was built by Box-Hunter with $k = 3$, $n_0 = 6$ to build the mathematical relationship between y_1, y_2, y_3 and x_1, x_2, x_3 . Number of experiment in this method was determined as the follow equation:

$$N = 2^k + 2 \cdot k + n_0 = 2^3 + 2 \cdot 3 + 6 = 20 \quad (5)$$

In order to the experimental matrix is orthogonal, α was determined as follow:

Table 1: Level of technological parameters in the experimental design

Parameters	Levels					Deviation ΔZ_i
	$\alpha(-1.682)$	Low -1	Central 0	High +1	$+\alpha(1.682)$	
x_1 (%)	1.69	2	2.5	3.0	3.34	0.5
x_2 (h)	13.90	18	24	30	34.09	6
x_3 ($^{\circ}$ C)	-18.81	-15	-10	-5	-1.59	5

Table 2: Matrix of Box-Hunter's secondary orthogonal experimental method

N	x_0	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	x_1^2	x_2^2	x_3^2	y_1	y_2	y_3
1	+	+	+	+	+	+	+	+	+	+	42.10	22.10	56.70
2	+	-	+	+	-	+	+	+	+	+	37.29	19.10	52.30
3	+	+	-	+	-	+	-	+	+	+	42.56	21.90	58.90
4	+	-	-	+	+	-	-	+	+	+	39.50	23.65	66.20
5	+	+	+	-	+	-	-	+	+	+	38.29	18.30	87.20
6	+	-	+	-	-	+	-	+	+	+	35.10	19.50	67.80
7	+	+	-	-	-	-	+	+	+	+	37.74	22.23	64.30
8	+	-	-	-	+	+	+	+	+	+	42.9	23.01	87.60
9	+	$+\alpha$	0	0	0	0	0	α^2	0	0	42.09	22.09	57.70
10	+	$-\alpha$	0	0	0	0	0	α^2	0	0	38.26	23.30	66.70
11	+	0	$+\alpha$	0	0	0	0	0	α^2	0	38.05	22.30	65.40
12	+	0	$-\alpha$	0	0	0	0	0	α^2	0	41.33	23.20	66.70
13	+	0	0	$+\alpha$	0	0	0	0	0	α^2	40.87	22.30	55.20
14	+	0	0	$-\alpha$	0	0	0	0	0	α^2	37.50	15.40	56.70
15	+	0	0	0	0	0	0	0	0	0	42.16	18.60	80.80
16	+	0	0	0	0	0	0	0	0	0	41.30	17.90	82.60
17	+	0	0	0	0	0	0	0	0	0	41.80	18.50	88.30
18	+	0	0	0	0	0	0	0	0	0	41.50	18.60	86.70
19	+	0	0	0	0	0	0	0	0	0	42.08	18.50	87.50
20	+	0	0	0	0	0	0	0	0	0	42.29	19.80	88.90

$$\alpha = 2^{k/4} = 1.682 \tag{6}$$

$$x_j \text{ is variables of objective function } j = 1 \div 3 \tag{7}$$

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 - 15 to -5° C

With limited domain is:

$$\Omega_X = (-1.682 \leq x_1, x_2, x_3 \leq 1.682) \tag{8}$$

From the conditions of technology, experimental process as well as production process, the range of the factors effect to the objectives in this research was presented in the Table 1.

The experiment was carried out with the level of technological parameters in Table 1 and experimental planning matrix (Box-Hunter) in Table 2. The results of determining the value of the objectives at different levels were presented in Table 2.

From the mathematical model and experimental result, the coefficients in the regression equation was determined. Subsequently, these coefficients were checked about the significance by Student standard and about the compatible of the regression equation by Fisher standard. There was the regression equation: recovery efficiency of DHA (y_1), recovery efficiency of EPA (y_2) and recovery efficiency of Omega-3 (y_3):

$$y_1 = 41.804 + 0.903x_1 - 1.129x_2 + 0.958x_3 + 1.262x_1x_2 + 1.23x_1x_3 - 0.604x_1^2 - 0.775x_2^2 - 0.954x_3^2 \tag{9}$$

$$y_2 = 18.634 - 0.974x_2 + 1.121x_3 + 1.359x_1^2 + 1.378x_2^2 \tag{10}$$

$$y_3 = 85.458 - 5.514x_3 + 6.8x_1x_2 - 6.695x_1^2 - 5.334x_2^2 - 8.906x_3^2 \tag{11}$$

The above equation showed the close relationship between the recovery efficiency of DHA, EPA, omega-3 and the parameters x_1, x_2, x_3 . When x_1, x_2, x_3 change, the function y_1, y_2, y_3 will change, respectively.

Building the single objective optimization: Effects of x_1, x_2, x_3 on objective functions y_1, y_2 and y_3 , described the enrichment progress of DHA, EPA and omega-3. It can be clearly seen that the single objective optimization problem was formed. Solve the single objective optimization problem means finding the root $x = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \in \Omega_X$ with $j = 1 \div 3$ for $y_{1max} = y_1(x_1^{1opt}, x_2^{1opt}, x_3^{1opt})$; $y_{2max} = y_2(x_1^{2opt}, x_2^{2opt}, x_3^{2opt})$; $y_{3max} = y_3(x_1^{3opt}, x_2^{3opt}, x_3^{3opt})$. Excel Solver software was used to find the maximum value of objective functions that was presented by Table 3.

The results of single objective optimization have showed that maximum level of y_1, y_2, y_3 was $y_{1max} =$

Table 3: Roots of the single objective optimization problems

Objective j	y_j	x_1^{jopt}	x_2^{jopt}	x_3^{jopt}
1	42.19	-0.048	-0.819	-0.022
2	29.88	-1.68	-1.68	1.68
3	86.03	-0.246	-0.124	-0.323

Table 4: The result determine the fish oil product

Order	Parameters	Content (%)
1	DHA	1.40
2	EPA	1.20
3	Content of omega-3	3.38
4	Recovery efficiency y_1 (DHA)	42.88
5	Recovery efficiency y_2 (EPA)	21.90
6	Recovery efficiency y_3 (omega-3)	85.50

42.19; $y_{2max} = 29.88$; $y_{3max} = 86.03$. The results were completely compatible with experimental data in Table 4. However, the single objective optimization problems (9, 10, 11) has no same root to satisfy $y_1 = y_{1max}$; $y_2 = y_{2max}$; $y_3 = y_{3max}$, due to $(x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \neq (x_1^{kopt}, x_2^{kopt}, x_3^{kopt})$ with $j, k = 1 \div 3, j \neq k$. Therefore, the root of each single objective optimization problem is not a same root of multi-objective optimization problem $y^{UT} = (y_{1max}, y_{2max}, y_{3max}) = (42.19; 29.88 ; 86.03)$. To solve this problem, need to build and solve the multi-objective problem which was presented in next section.

Building the multi-objective optimization problem:

Due to the parameters affected the objective functions in the same technological subject; therefore, the effects of x_1, x_2, x_3 on all objective functions y_1, y_2, y_3 were determined simultaneously. As a consequence, it could be seen that in the research of determining the recovery efficiency, the multi-objective optimization problem had appeared. Thus, the multi-objective optimization problem was written as follow: finding the root $x = (x_1^{opt}, x_2^{opt}, x_3^{opt}) = \{-1.682 \leq x_1, x_2, x_3 \leq 1.682\} \in \Omega_x$ with $j = 1 \div 3$ in order to:

$$\begin{cases} I_{jmin} = I_j(x_1^{opt}, x_2^{opt}, x_3^{opt}) = \text{Min}\{I_j(x_1, x_2, x_3)\} \\ \forall x = (x_1, x_2, x_3) \in \Omega_x; j = 1 \div 3 \end{cases} \quad (12)$$

Therefore, the multi-objective optimization problem (12) need to be solved to find optimal Pareto root (x_1S, x_2S, x_3S) so that the distance between optimal Pareto efficiency IPS and utopian point $I^{UT} = (I_1 \text{ min}, I_2 \text{ min}, I_3 \text{ min})$ was the closest. The results obtained were used to set up technological mode of the enriching process of DHA, EPA and omega-3 of pangasius fish oil to apply for industrial production process.

According to Canh (2004); Luc and Hai (2008), to find root of multi-objective optimization problem, the utopian method should be applied. This method proposed S-optimal combination criterion, it is distance between the point in optimal Pareto efficiency set and the utopian point and can be written as follow:

$$S(x) = \left[\sum_{j=1}^m S_j^2(x) \right]^{\frac{1}{2}} = \left[\sum_{j=1}^m (y_j(x) - y_j \text{ max})^2 \right]^{\frac{1}{2}} \quad (13)$$

It can be seen $S(x)$ was the distance between the point $y(x)$ to utopian point y^{UT} :

$$\forall x = (x_1, x_2, x_3) = \{-1.682 \leq x_1, x_2, x_3 \leq 1.682\} \in \Omega_x \quad (14)$$

If $S(x)$ was chosen as objective function, the multi-objective optimization problem (13) would be present as follow: to find optimal Pareto root $xS = (x_1S, x_2S, x_3S) = \{-1.682 \leq x_1, x_2, x_3 \leq 1.682\} \in \Omega_x$ in order to:

$$\begin{cases} S_{min} = S(x_1S, x_2S, x_3S) = \text{Min}\{S(x_1, x_2, x_3)\} = \text{Min}\left\{\sum_{j=1}^3 (I_j - I_{jmin})^2\right\}^{1/2} \\ \forall x = (x_1S, x_2S, x_3S) = \{-1.682 \leq x_1, x_2, x_3 \leq 1.682\} \in \Omega_x \end{cases} \quad (15)$$

The multi-objective optimization problem were solved by mesh method programed in Matlab 7.1 software. The result had found roots as follow:

$$S_{min} = 9.512 \text{ at } x_1S = -0.8; x_2S = -0.898; x_3S = -0.208 \quad (16)$$

Substitute (16) into (9), (10) and (11) to find the optimal Pareto root as follow:

$$y_1PS = 41.86; y_2PS = 21.26; y_3PS = 82.52$$

Change (16) into real root: The ratio of urea-to-fish oil $x_1 = 2/1$; $x_2 = 18.6$ hours and $x_3 = -11^\circ\text{C}$. Under these conditions, the recovery efficiency reached to $y^{UT} = (y_{1max}, y_{2max}, y_{3max}) = (41.86; 21.26; 82.52)$.

With optimal Pareto roots $x_1S = -0.80$; $x_2S = -0.897$; $x_3S = -0.208$ (the real roots were the ratio of urea-to-fish oil $x_1 = 2/1$, $x_2 = 18.6$ h and $x_3 = -11^\circ\text{C}$, respectively) of the multi-objective optimization problem (12) or (15), the mathematical model about the urea complexation process of Pangasius fish oil was built with optimal Pareto efficiency IPS = (I_1PS, I_2PS, I_3PS) had the nearest distance with $y^{UT} = (y_{1max}, y_{2max}, y_{3max}) = (41.86; 21.26; 82.52)$.

The results showed that optimal Pareto roots was the best root. Therefore, it could be used to establish technological mode for the enrichment process of DHA, EPA and omega-3 of pangasius fish oil.

Experiment confirm optimal pareto root: When the pangasius fish oil experiment was carried out at optimal Pareto root with the ratio of urea-to-fatty acid of 2/1, complexation temperature of -11°C and complexation time of 18.6 h, the result showed that $y_1 = 42.88$; $y_2 = 21.90$ và $y_3 = 85.50$. In comparison with optimal Pareto efficiency ($y_1PS = 41.86$; $y_2PS = 21.26$; $y_3PS = 82.52$), the optimal Pareto roots were completely compatible with the experimental data.

The simulation of the mathematical models of the objective functions in 3D was performed in Fig. 1 to 4.

Determine the enrichment efficiency: After select the factors, which affect the enrichment process of DHA and EPA, then carried out to determine the content of DHA, EPA and Omega-3 ($C_{18:3}$; $C_{20:5}$; $C_{22:6}$) in fish oil, the result is showed as below:

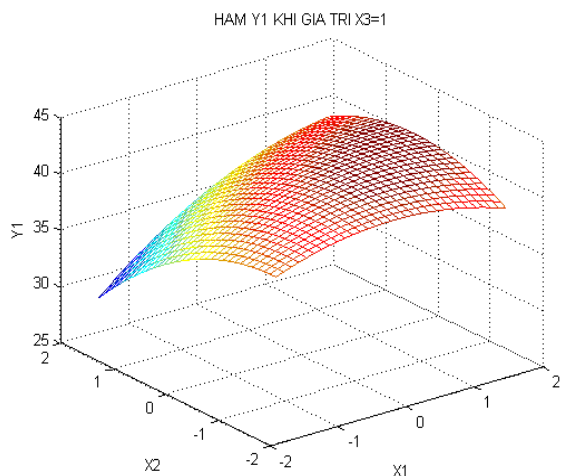


Fig. 1: Response surface of function y1 – efficiency of DHA

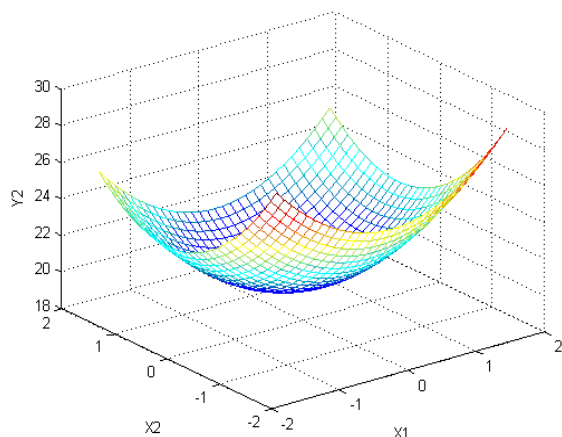


Fig. 2: Response surface of function y2 – efficiency of EPA

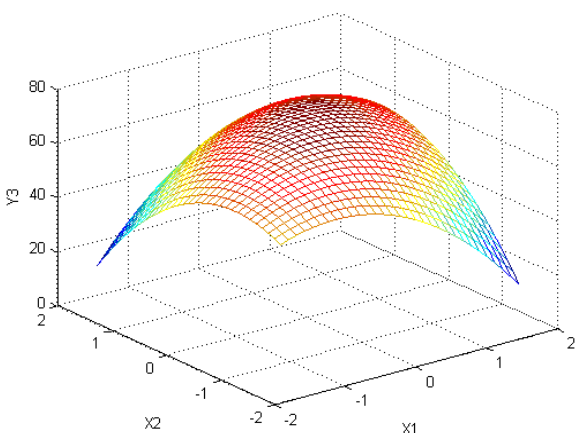


Fig. 3: Response surface of function y3 – efficiency of omega-3

From the above result, determine:
Enrichment efficiency of DHA:

$$HDHA = \frac{1.40 \times 4.288}{0.5 \times 10} \cong 120.06\%$$

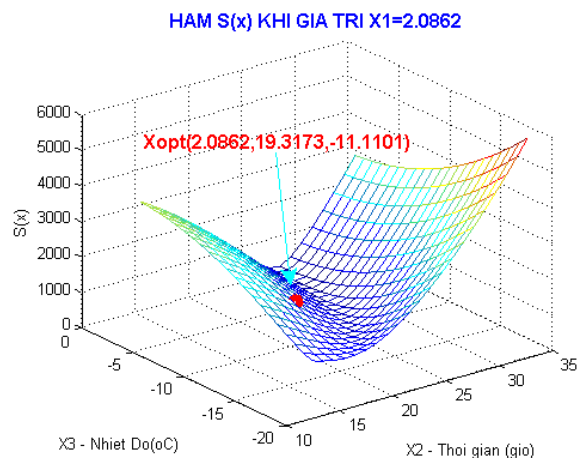


Fig. 4: Response surface of combinational function S(x) at x1 = 2/1

Enrichment efficiency of EPA:

$$H_{EPA} = \frac{1.20 \times 2.19}{0.45 \times 10} \cong 58.4\%$$

Enrichment efficiency of Omega-3:

$$H\omega_3 = \frac{3.38 \times 8.55}{1.61 \times 10} \cong 179.5\%$$

The results showed that the content of DHA reached a high recovery efficiency of 120.06% while the content of EPA and omega-3 reached the recovery efficiency of 58.4 and 179.5%, respectively. This proves the active of enrichment properties has increased significantly, particularly contents of DHA, omega-3 while EPA has the metabolism which arranged structure has reached a saturation level, hence reached the lower enrichment. The results of the study demonstrated the metabolism enrichment in fish oil has increased, the molecular has been arranged and increased polyunsaturated fatty acid. Contents of DHA and EPA omega-3 after the enrichment has been increased significantly. At the same time, the results determine that the optimal pareto and optimal pareto efficiency were totally compatible with the experimental data.

CONCLUSION

Optimization confirmed a close relationship between technology and mathematics. Shedding light on the technology and equipment under systematic approach is a modern approach by mathematical thinking. It created very favorable conditions for the system partition according to the quantities and relations, in order to control the control technology. From the above results show that the mathematical model has a clear expression of the enrichment of DHA, EPA and Omega-3 in fish oil extraction technology.

The results showed that optimal pareto root is the best root in the ratio of urea-to-fish oil of 2/1; complexation temperature of -11°C and complexation time of 18.6 h. It has resulted in the concentrations of DHA, EPA and Omega-3 enrichment process achieved 120.06, 58.4, 179.5%, respectively and this is the basis for establishing technological mode for the enrichment of DHA and EPA omega-3 in fish oil.

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