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Optimization of the Refining Process for a Fraction Rich in Crude Common Pony Fish (Leiognathus equulus) Oil as a By-product of Fish Protein Hydrolysate Processing Using The Response Surface Method

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ABSTRACT

The production of fish protein hydrolysate from common pony fish yields a fraction rich crude fish oil as a by-product. To utilize this by-product, refining is necessary to obtain common pony fish oil. This research aims to use a fraction rich crude fish oil from fish protein hydrolysate by-products to obtain fish oil and to determine the optimal conditions for the refining process using the response surface method (RSM) in the degumming, neutralization, and bleaching processes. The experimental design used was the Box–Behnken design, with the responses used to determine the optimum conditions at the degumming stage were water content and total dissolved solids. The response used to determine the optimum conditions at the neutralization stage was the refining factor, and at the bleaching stage was color (L, a, b*). Parameters studied for each purification process include heating temperature (50-80°C), contact time between fraction rich crude fish oil with auxiliary materials (10-20 minutes) and the length of time for centrifugation (5-15 minutes) with a rotation speed of 10,062 G. The optimal conditions obtained in the degumming, neutralization, and bleaching processes for heating temperature, contact time between fraction rich crude fish oil with auxiliary materials, and centrifugation time, respectively, were 50°C, 10 min, 5 min; 50°C, 20 min, 5 min; and 80°C, 10 min, 15 min. Verification of the optimum conditions resulted in a free fatty acid value of 8.25% ± 0.01%, an acid value of 1.87 ± 0.02 mg KOH/g oil, a peroxide value of 1.04 ± 0.01 meq/Kg, an anisidine value of 11.11 ± 0.01 meq/Kg, a total oxidation value of 13.21 ± 0.01 meq/Kg and water content 6.052 ± 0.02 %. These results indicate a reduction in free fatty acids, acid number, peroxide number, anisidine number and water content by 66%, 90%, 73.5%, 61%, 63%, and 92% respectively. Our results showed that the purified fraction rich crude fish oil has met the SNI standards in parameter acid number, peroxide number, iodine number, anisidine number, and total oxidation value. The purification process that has been carried out can improve the quality fraction rich crude fish oil, but further processing still needs to be carried out to reduce water content and free fatty acid value.*

Keywords: *common pony fish, fraction rich of crude fish oil, purification, degumming, neutralization, bleaching, and RSM.*

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INTRODUCTION

The purpose of this study is to optimize the refining process of fraction rich crude fish oil using the response surface method (RSM) to determine the optimum treatment that can be performed in the fraction rich crude fish oil refining process to produce common pony fish oil according to IFOMA oxidation parameter standards and SNI 8467: 2018. Fraction rich crude fish oil is waste from the production of fish protein hydrolyzate which still contains carbohydrates, proteins, fats, and other nutrients that still have the potential to be utilized. According to Bija (2017), the fish oil refining process can be performed in three stages, namely degumming (removal of gum/slime), neutralization (removal of free fatty acids in oil using alkaline bases), and bleaching. The degumming process with NaCl converts non-hydratable phospholipids (which cannot be hydrated) into hydratable phospholipids (which can be hydrated) so that they can be easily separated in oil (Sirait, 2021). NaOH was used in the neutralization stage due to its cost-effectiveness and efficiency. The neutralization step aims to reduce the free fatty acid content in fish oil and thicken unwanted substances (Sari et al., 2015). The bleaching stage usually undergoes an adsorption process with adsorbents. Results of previous studies show that activated charcoal can absorb color components and separate free fatty acids from fish oil. Free fatty acid content, the amount and type of adsorbent used, temperature, and treatment time affect the color and turbidity of the oil. To find out the optimum point of the fraction rich crude fish oil refining process with the right output, the fraction rich crude fish oil refining process needs optimization.

Previous research on fish oil refining process optimization used the RSM. RSM is one of the methods that has been successfully applied in various process optimization research. Research by (Salia et al., 2019), (Sadida, 2020), and (Sirait, 2021) used the RSM in the fish oil refining process. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing responses, which are influenced by several variables to optimize the response. The optimum solution in RSM can be selected for maximum, minimum, and conditions along the upper limit and lower limit (Montgomery, 2012). The RSM offers the advantage of not requiring extensive experimental data and prolonged time (Sirait, 2021). The use of RSM from the Design Expert 13 program is expected to produce an optimal process in the fraction rich crude fish oil refining, tailored to comply with IFOMA and SNI 8467: 2018 (Sadida, 2020).

RESEARCH METHODS

The materials needed for the refining stage are fractions rich crude fish oil obtained from PT. Berikan Teknologi Indonesia, NaCl (pro analysis), NaOH (pro analysis), activated charcoal (pro analysis), potassium iodide (pro analysis), acetic acid (pro analysis), sodium thiosulfate (pro analysis), chloroform (pro analysis), potassium hydroxide (pro analysis), starch indicator, p-anisidine, phenolphthalein, cyclohexane, Wijs solution, 95% alcohol, and distilled water. The tools used at this stage are a centrifugator, centrifuge tube, analytical digital scales, magnetic stirrer, stirrer, beaker, filter paper, and funnel. Tools used in the analysis process are analytical digital scales, clamps and statives, burets, beakers, measuring cups, drop pipettes, and erlenmeyer flasks.

The research conducted involved two stages, namely preliminary research and main research. Preliminary research is carried out to determine the characteristics of fraction rich crude fish oil before refining so that the comparison can be seen before and after refining. The preliminary research encompasses the assessment of parameters such as free fatty acids, peroxide number, anisidine number, total oxidation and water content. The main research was carried out through three stages of fraction rich crude fish oil purification, namely degumming using NaCl, neutralization using NaOH, and bleaching using activated charcoal. The concentrations used were NaCl at 10% w/v, NaOH at $18°Be$, and activated charcoal at 9.4% w/v of the fraction rich crude fish oil. In the refining process, the combination of heating temperature (50-80°C), contact time between fraction rich crude fish oil with auxiliary materials (10-20 minutes) and the length of time for centrifugation (5-15 minutes) with a rotation speed of 10,062 G were adjusted based on the recommendations from the Design Expert 13 application using the RSM. The objective function used to conduct optimization in this research is to minimize all responses related to parameters of oil damage and optimize the yield and fat content of fish oil produced.

The goal or objective in degumming purification optimization in the response of water content and total soluble solids are to achieve the lowest or smallest water content and total soluble solids. The goal or objective in the neutralization purification process, focusing on the refining factor response, is to achieve the lowest or smallest refining factor. And the goal or objective in optimizing the bleaching purification process, focusing on the color response, are to achieve the highest color (L) or the largest value, the lowest color (a*) or the smallest value, and the lowest color value (b*) or the smallest value.

RESULTS AND DISCUSSION

Preliminary research: characteristics of fraction rich crude fish oil . Fish oil is the fatty component from a fish's body tissue that has been extracted in liquid (oil) form. Fish oil by-products contain glyceride and non-glyceride components. The main components are triglycerides (the largest component), diglycerides, and monoglycerides. Glycerides are components of the saponifiable fraction, which means that when alkali is added, soap can be formed. Other components of unsaponifiable oils are phospholipids, sterols, and waxes (Estiasih, 2009). In this study, fraction rich crude fish oil was obtained from industrial by-products from PT. Berikan Teknologi Indonesia. Before the main research, a preliminary research was carried out first to test the characteristics of fraction rich crude fish oil.

The analysis results in Table 1 show that fraction rich crude fish oil has low quality. The analysis parameters, which include acid number, iodine number, anisidine number, fat content, free fatty acids, saponification number, total oxidation and water content do not meet IFOMA and SNI standards; only the peroxide number meets the standards. It can be caused by the oil being exposed to air, using high temperatures during processing, and storing fish oil at room temperature. Fish oil storage at -18 ℃ has a longer shelf life, twice as long as storage at 4℃. Storage exposed to oxygen accelerates oxidation. The unsaturated fatty acids contained in fish oil are easily oxidized when exposed to oxygen and form peroxide compounds. Peroxide compounds can be degraded into p-anisidine compounds. The higher the anisidine and peroxide numbers, the higher the total oxidation number. Optimization of the purification process is carried out in the degumming process, neutralization, and bleaching purification process

Table 1. Results of Fraction Rich Crude Fish Oil Analysis

No	Analysis Parameters	Results	Standard (IFOMA, 1998)	Standard (SNI, 2018)	Description
1	Acid number (mg KOH/g)	18.29 ± 0.05	≤ 3	-	Not Suitable
2	Peroxide number (mEq/Kg)	3.93 ± 0.01	3-20	3-5	Suitable
3	Iodine number (g iod/100g oil)	7.24 ± 0.02	-	< 2.21	Not Suitable
4	Anisidine number (mgEq/Kg)	28.21 ± 0.01	≤ 20	≤ 20	Not Suitable
5	Fat content (%)	3.55 ± 0.01	-	-	-
6	Free fatty acids (%)	24.54 ± 0.02	1-7	1-2	Not Suitable
7	Saponification number (mg KOH/g fat)	39.71 ± 0.01	-	-	-
8	Total oxidation value	36.07 ± 0.02	≤ 26	≤ 26	Not Suitable
9	Water content (%)	76.80 ± 0.12	0.5-1.0	-	Not Suitable

Main research: refining of fraction rich crude fish oil. Following the preliminary research, which involved analyzing the quality of fraction rich of crude fish oil, the main research was carried out, that is the fish oil purification. The aim was to determine the characteristics and compare fraction rich crude fish oil quality before and after refining. The optimization of the refining process was carried out in three stages, namely, optimization of the degumming refining process, followed by optimization of the neutralization refining process, and finally optimization of the bleaching refining process. The purification process optimization was carried out in stages, starting with degumming, followed by neutralization and bleaching.

Degumming purification process. In the analysis of the 13 runs of the degumming refining process, the water content test on fraction rich crude fish oil has the lowest water content of 11.25% in the 11th run and the highest water content of 23.05% in the 7th run. For the total soluble solids test, the lowest %Brix was 2.85% in the 4th run, and the highest %Brix was 4.95% in the 1st run.

Table 2. Results of Response Analysis of Water Content and Total Dissolved Solids (TDS) in the Degumming Purification Process

Run	Factor 1 A: heating temperature(°C)	Factor 2 B: contact time of fraction rich crude fish oil with NaCl (minute)	Factor 3 C: centrifugation time (minute)	Response 1 water content (%)	Response 2 TDS (%Brix)
1	50	15	5	16.25	4.95
2	65	10	5	18.04	4.85
3	65	15	10	17.22	3.22
4	65	20	15	17.54	2.85
5	80	15	15	12.00	2.97
6	80	10	10	12.14	3.53
7	80	20	10	23.05	3.44
8	50	10	10	15.65	3.62
9	50	15	15	16.32	2.89
10	65	20	5	17.05	4.05
11	80	15	5	11.25	4.05
12	50	20	10	16.55	3.85
13	65	10	15	17.25	2.95

Water content. Moisture content is one of the most important chemical laboratory tests used in the food industry to determine the quality and resistance of food, including oil samples. The linear regression equation is used to obtain the optimum variable affecting the water content in fraction rich of crude fish oil during the degumming refining process. The coefficients of the linear model of water content are as follows: $16.18 - 0.7913A + 1.39B + 0.0650C$. Based on the equation obtained, variables B and C will increase the value of water content marked with a positive value, with the contact time of fractions rich crude fish oi with NaCl (B) greatly affecting the value of water content. Conversely, variable A will reduce the value of water content characterized by a negative value, which means that the heating temperature factor (A) does not really affect the value of water content.

The effect of temperature and heating time factors in the water content response can be seen in the contour and 3D surface graph models. In the figure 1, increasing shades of blue show areas with low water content response values, while increasing shades of red show areas with high water content response values. As observed from the 3D surface graph of the water content response, the longer the heating time, the higher the water content, and the lower the heating temperature, the higher the water content, and vice versa. The goal or objective in the degumming purification optimization on the water content response is to achieve the lowest or smallest water content. Hence, the optimization result point on the 3D surface graph of the water content response is at the highest heating temperature and the shortest heating time.

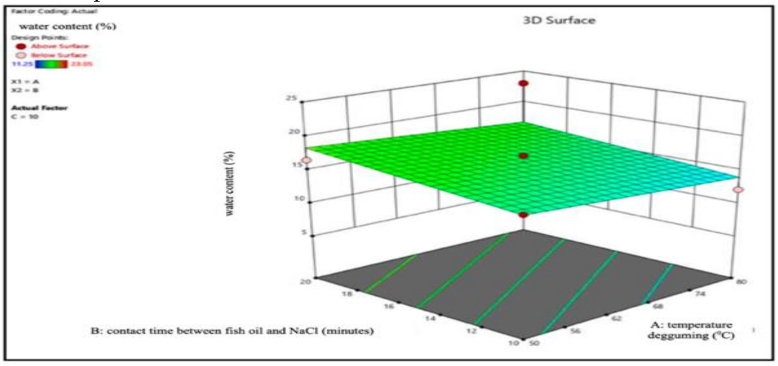

Figure 1. 3D Surface Graph of Water Content Response to Temperature with Contact Time Between Fraction Rich Crude Fish Oil and NaCl

Total Dissolved Solids. Total dissolved solids (TDS) are the dissolution of solid substances, either in the form of ions, compounds, or colloids in water. TDS are the total elements or mineral elements dissolved in a solution, which is usually caused by inorganic materials in the form of ions commonly found in water. If the TDS increase, the hardness of the water will also increase. TDS measurements can be made using a refractometer (Rivaldi et al., 2019). The linear regression equation is used to obtain the optimum variables on the value of total soluble solids in fraction rich of crude fish oil degumming refining process follows $3.63 - 0.1650A - 0.0950B - 0.7800C$.

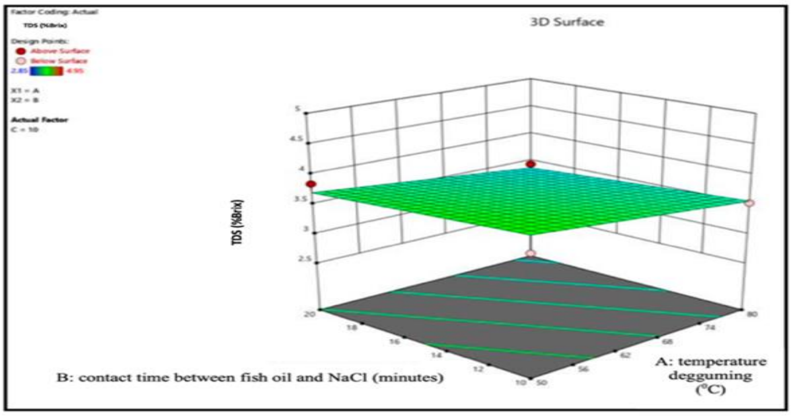

Figure 2. 3D Surface Graph of Total Dissolved Solids Response to Temperature; contact time between fraction rich crude fish oil and NaCl

Based on the equation obtained, variables A, B, and C will reduce the value of TDS marked with negative values, that is all factors that have a little effect on the value of TDS. In contrast, there are no variables that increase the value of TDS, which means that there are no factors that greatly affect the value of TDS. The effect of temperature and heating time factors on the response of TDS can be seen in the contour and 3D surface graph models. In the figure, increasing shades of blue show areas with low TDS response values, while increasing shades of red show areas with high TDS response values. As observed from the 3D surface graph of the total soluble solids response (figure 2), the longer the heating time, the lower the total soluble solids, and the lower the heating temperature, the higher the total soluble solids, and vice versa. The goal or objective in degumming purification optimization in the response of total soluble solids is to achieve the lowest or smallest total soluble solids. Hence, the optimization result point on the 3D surface graph of the total soluble solids response is at the highest heating temperature and the longest heating time.

Degumming Optimization Results. The optimization results obtained from the Design Expert software using RSM obtained optimization conditions for the degumming purification process: heating temperature at 50°C, contact time between fraction rich crude fish oil and NaCl at 10 minutes, and centrifugation time at 5 minutes with a rotation speed of 10,062 G. The optimization results are seen from the highest desirability in the table 3, the desirability obtained is 0.825.

Table 3. Optimization Results of Degumming Stage Purification Using Design Expert Software

Selected	Recommendation 1	Recommendation 2
Heat temperature	50°C	50°C
Contact time between fraction rich crude fish oil and NaCl	10 minutes	10 minutes
Centrifugation time	5 minutes	5 minutes
Water content	15.51%	15.50%
TDS	4.67% Brix	4.67% Brix
Desirability	0.825	0.824

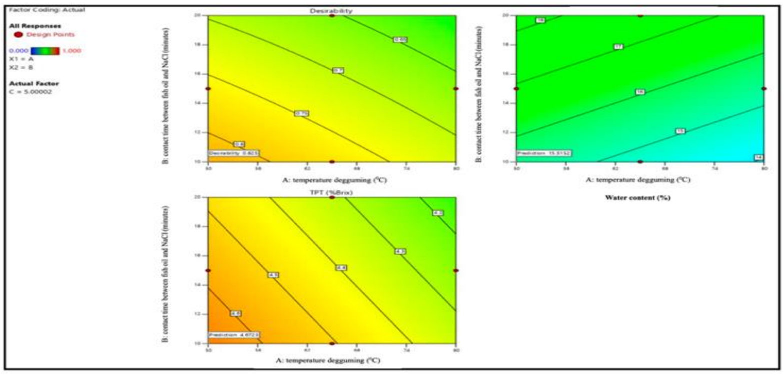

Figure 3. Contour Graph of all Degumming Purification Responses

Neutralization purification process. In the analysis results of the 13 runs of the neutralization refining process, refining factor data on fraction ric hcrude fish oil have the lowest data of 0.542 in the 1st run and the highest data of 2.294 in the 6th run. The refining factor indicates the efficiency of neutralization, which is the ratio between the total loss due to neutralization and the amount of free fatty acids in crude fat. The smaller the refining factor value, the higher the neutralization efficiency (Ketaren, 2012).

Table 4**.** Results of Refining Factor Response Analysis in the Neutralization Purification Process

R u n	Factor 1 A: heating temperature(°C)	Factor 2 B: contact time of fraction rich crude fish oil with NaOH (minute)	Factor 3 C: centrifugation time (minute)	Response 1 Refining Factor
1	50	15	5	0.542
2	65	10	5	1.578
3	65	15	10	1.501
4	65	20	15	1.601
5	80	15	15	2.126
6	80	10	10	2.294
7	80	20	10	2.188
8	50	10	10	0.863
9	50	15	15	0.761
10	65	20	5	1.351
11	80	15	5	2.119
12	50	20	10	0.766
13	65	10	15	1.782

The linear regression model equation for the refining factor response as follows: $1.50 + 0.7244A - 0.0764B + 0.0850C$. Based on the equation obtained, variables A and C will increase the refining factor value marked with a positive value, with the heating temperature factor (A) greatly affecting the refining factor value. Variable B will decrease the refining factor value marked with a negative value, which means that the contact time of fraction rich crude fish oil with NaOH (B) does not really affect the refining factor value.

As observed from the 3D surface graph (figure 4) of the refining factor response, the longer the heating time, the lower the refining factor value, and the lower the heating temperature, the lower the refining factor value, and vice versa. The goal or objective in the neutralization purification process, focusing on the refining factor response, is to achieve the lowest or smallest refining factor. Hence, the optimization result point on the 3D surface graph of the refining factor response is at the lowest heating temperature with the longest heating time.

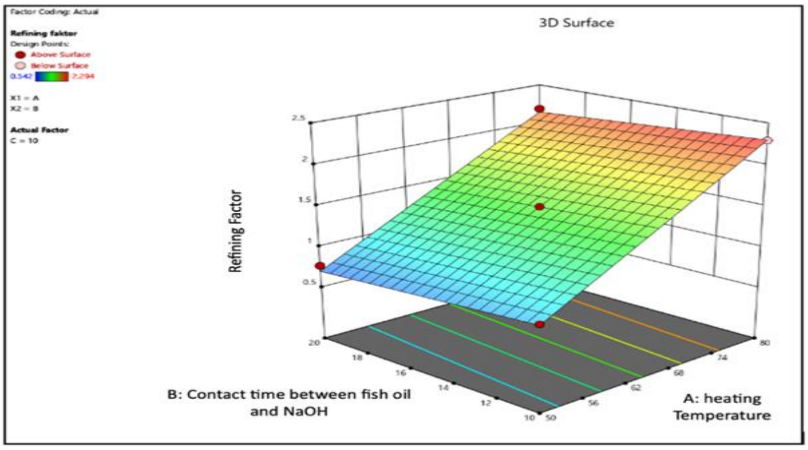

Figure 4. 3D Surface Graph of Refining Factor

Neutralization Optimization Results. The optimization results obtained from the Design Expert software using RSM obtained optimization conditions for the neutralization purification process are as follows: heating temperature at 50°C, contact time between fraction rich crude fish oil and NaOH at 20 minutes, and centrifugation time at 5 minutes with a rotation speed of 10,062 G. The optimization results are seen from the highest desirability in the table 5, the desirability obtained is 0.965.

Table 5. Neutralization Purification Optimization Results

Selected	Recommendation 1	Recommendation 2	Recommendation 3
Heat temperature	50°C	50°C	50°C
Contact time between fraction rich crude fish oil and NaOH	**20 minutes**	20 minutes	20 minutes
Centrifugation time	**5 minutes**	5 minutes	5 minutes
Refining faktor	**0.612**	0.611	0.610
Desirability	**0.965**	0.960	0.959

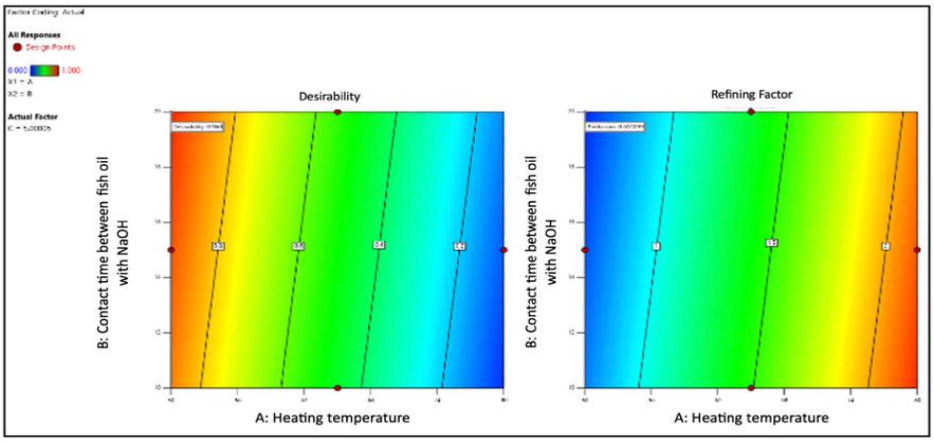

Figure 5. Contour Graph of All Neutralization Purification Responses

Bleaching purification process . In the analysis results of the 13 runs of the bleaching refining process, the color test on fraction rich crude fish oil has the lowest L (light) color of 35.97 in the 9th run and the highest of 52.85 in the 7th run. For the color −a* (green) in fraction rich crude fish oil, the lowest was −0.25 in the 12th run and the highest was −0.49 in the 5th run. For +b* (yellow) color in fraction rich crude fish oil, the lowest was 3.22 in the 8th run and the highest was 6.25 in the 7th run.

Table 6. Results of Color Response Analysis Fraction Rich Crude Fish Oil in the Bleaching Purification Process

Run	Factor 1 A: heating temperature (°C)	Factor 2 B: contact time of fraction rich crude fish oil with activated charcoal (minute)	Factor 3 C: centrifugation time (minute)	Color		
				L	a*	b*
1	50	15	5	37.77	-0.32	3.68
2	65	10	5	42.85	-0.35	4.48
3	65	15	10	44.86	-0.46	5.05
4	65	20	15	41.98	-0.36	5.65
5	80	15	15	51.25	-0.49	6.02
6	80	10	10	50.98	-0.46	5.99
7	80	20	10	52.85	-0.43	6.25
8	50	10	10	36.84	-0.28	3.22
9	50	15	15	35.97	-0.30	3.35
10	65	20	5	45.04	-0.47	4.49
11	80	15	5	51.04	-0.42	6.08
12	50	20	10	36.66	-0.25	3.29
13	65	10	15	46.85	-0.45	5.25

Color (L). Color is the impression obtained by the eye from light reflected by objects that are subjected to light. Color is part of the light that is transmitted or reflected. There are three important elements that define color, namely objects, eyes, and light elements. Good and healthy oil is usually clear, and the golden-yellow color of the oil appears due to the content of beta carotene.The linear regression model equation for the color response Color (L) = 44.23 + 7.36A − 0.1238B − 0.0813C + 0.5125AB + 0.5025 AC − 1.77 BC. Based on the equation obtained, variables A, AB, and AC will increase the color value (L) marked with a positive value, with the heating temperature factor (A) greatly affecting the color value (L). Variables B, C, and BC will decrease the color value (L) marked with negative values, which means that contact time of fraction rich crude fish oil with NaOH factor (B) and centrifugation time (C) do not really affect the color value (L). As observed from the 3D surface graph (figure 7) of color response (L), the longer the heating time, the lower the color value (L), and the lower the heating temperature, the lower the color value (L), and vice versa. The goal or objective in optimizing the bleaching purification process, focusing on the color response (L), is to achieve the highest color (L) or the largest value. Hence, the optimization result point on the 3D surface graph of the color response (L) is at the highest heating temperature and the longest heating time as well.

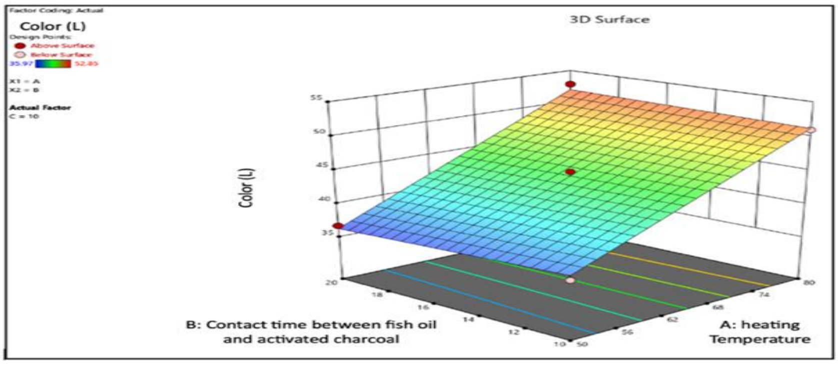

Figure **7**. 3D Surface Graph of Color Response (L) Against Heating Temperature with Contact Time of Fraction Rich Crude Fish Oil with Activated Charcoal

Color (a*). The linear regression model equation for the response of TDS as follows: Color (a*) = −0.3877 − 0.0812A + 0.0038B − 0.0050C. Based on the equation obtained, variable B will increase the color value (a*) marked with a* positive value, with the contact time of fraction rich crude fish oil with NaOH factor (B) being quite influential on the color value (a*), whereas variables A and C will decrease the color value (a*) marked with negative values, which means that the heating temperature factor (a*) and centrifugation time (C) do not really affect the color value (a*). As observed from the 3D surface graph (figure 8) of color response (a*), the longer the heating time, the higher the color value (a*), and the lower the heating temperature, the higher the color value (a*), and vice versa. The goal or objective in the optimization of bleaching purification, focusing on the color response (a*), is to achieve the lowest color (a*) or the smallest value. Hence, the optimization result point on the 3D surface graph of the color response (a*) is at the highest heating temperature with the shortest heating time.

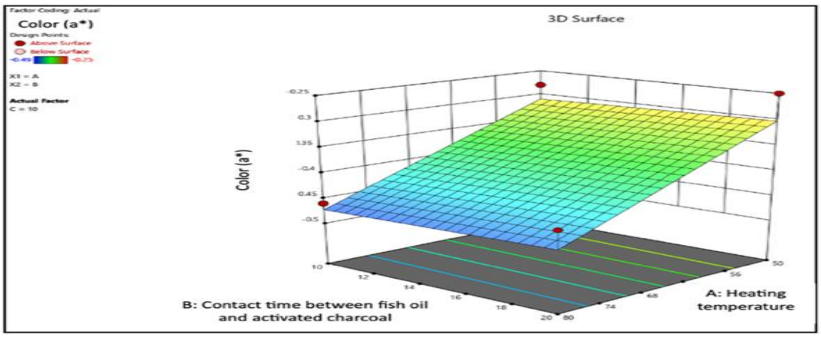

Figure 8. 3D Surface Graph of Color Response (a*) Against Heating Temperature; with Contact Time of Fraction Rich Crude Fish Oil with Activated Charcoal

Color (b*). The linear regression model equation for the color response (b) in the table is as follows: Color (b*) = 4.83 + 1.35A + 0.0925B + 0.1925C. Based on the equation obtained, variables A, B, and C will increase the color value (b*) marked with a positive value, with the heating temperature factor (A) greatly affecting the color value (b*). There are no variables that decrease the color value (b*), which means that all factors affect the color value (b*).

As observed from the 3D surface graph of color response (b*) in figure 9, the longer the heating time, the higher the color value (b*), and the lower the heating temperature, the lower the color value (b*), and vice versa. The goal or objective in the optimization of bleaching purification, focusing on the color response (b*), is to achieve the lowest color value (b*) or the smallest value. Hence, the optimization result point on the 3D surface graph of the color response (b*) is at the lowest heating temperature and the shortest heating time as well.

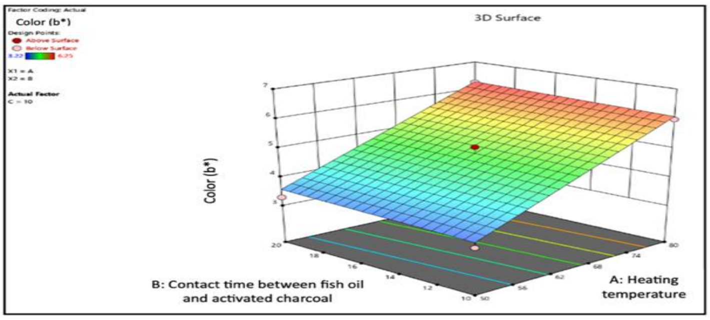

Figure 9. 3D Surface Graph of Color Response (b*) Against Heating Temperature with Contact Time of Fraction Rich Crude Fish Oil with Activated Charcoal

Bleaching Optimization Results. The optimization results obtained from the Design Expert software using RSM obtained optimization conditions for the bleaching purification process, namely heating temperature at 80°C, heating contact time between fraction rich crude fish oil and activated charcoal at 10 minutes, and centrifugation time at 15 minutes with a rotation speed of 10,062 G. The optimization results are seen from the highest desirability in the table 7, the desirability obtained is 0.983.

Table 7. Bleaching Purification Optimization Results

Selected	Recommendation 1	Recommendation 2
Heat temperature	80°C	80°C
Contact time between fraction richcrude fish oil and activated charcoal	10 minutes	10 minutes

Centrifugation time	15 minutes	15 minutes
Color (L)	53.383	53.348
Color (a*)	-0.478	-0.478
Color (b*)	6.281	6.283
Desirability	0.983	0.982

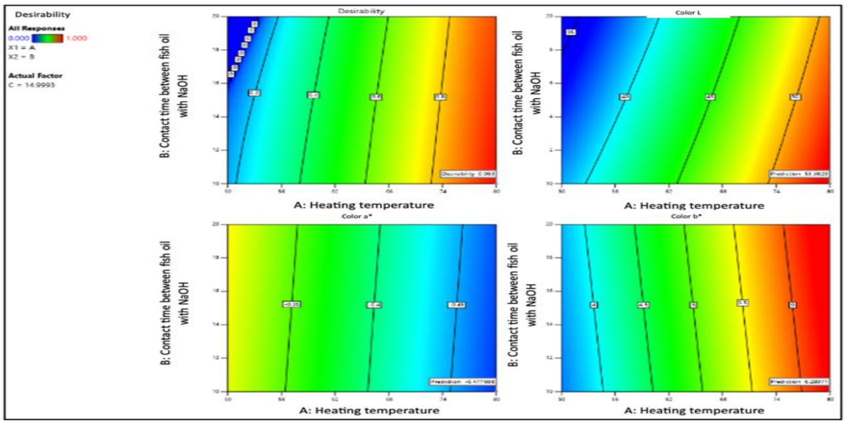

Figure 24. Contour Graph of All Bleaching Purification Responses

Purification process optimization verification. Verification of the response variable predictions is carried out after obtaining the results of simultaneous response optimization. The actual response value at this stage will be compared with the predicted response value provided by the design expert program. The design expert program provides a predicted response value followed by a 95% prediction interval (PI). The prediction interval is divided into 95% PI low and 95% PI high. PI low is the lowest value of the predicted interval (lower limit), while PI high is the highest value (upper limit). The values in the actual data column are obtained from the laboratory analysis results. In contrast, the values in the prediction mean and median column are obtained from the processing results of the Design Expert version 13 program.The following compares the results of the predicted response values provided by the design expert program with the analysis results after purification in the degumming purification process. Verification of response variable predictions is carried out after obtaining simultaneous response optimization results. The actual response value at this stage will be compared with the predicted response value given by the Design Expert program.

Table 8. Comparison of Predicted Response Value Results with Actual Value of Degumming Purification

Analysis	Predicted Mean	Predicted Median	95% PI low	Actual Data	95% PI high
Water content (%)	15.515	15.515	6.726	6.052	24.303
TDS (%Brix)	4.6723	4.6723	3.889	4.625	5.455

From the results of the table 8, the predicted response value on water content with the actual value of the analysis results is not considerably different. The actual data are still in the range of 95% PI low and 95% PI high, while the predicted response value on TDS with the actual value of the analysis is considerably different.

Table 9. Comparison of Predicted Response Value Results with Actual Value of Neutralization Purification

Analysis	Predicted Mean	Predicted Median	95% PI low	Actual Data	95% PI high
Refining factor	0.6121	0.6121	0.3205	0.781	0.9036

Table 9 is a comparison of the results of the predicted response value given by the Design Expert program with the actual value of the analysis results after purification in the neutralization purification process. From the results of the table 9, the predicted response value on the refining factor with the actual value of the analysis results is not considerably different. The actual data are still in the range of 95% PI low and 95% PI high. Hence, the optimization results factors, namely the heating temperature, contact time between fraction rich crude fish oil and NaOH, and centrifugation time, in the neutralization purification process given by the Design Expert program have provided optimal results because the refining factor data falls into the prediction range given by the program.

Table 10. Comparison of Predicted Response Value Results with Actual Bleaching Purification Values

Analysis	Predicted Mean	Predicted Median	95% PI low	Actual Data	95% PI high
Color L	44.2262	44.2262	42.094	48.22	46.358
Color a*	-0.38769	-0.38769	-0.5146	-0.28	-0.2607
Color b*	4.83077	4.83077	4.0452	4.41	5.6162

The following is a comparison of the results of the predicted response value given by the Design Expert program with the actual value of the analysis results after purification in the bleaching purification process. From the results of the table 10, the predicted response value on color (L) with the actual value of the analysis results is not considerably different, but the actual data exceeds the range of 95% PI low and 95% PI high. The predicted response value on color (a*) with the actual value of the analysis results is not considerably different. The actual data are still in the range of 95% PI low and 95% PI high. While the predicted response value in color (b*) with the actual value of the analysis results is not considerably different. The actual data are still in the range of 95% PI low and 95% PI high. Hence, the optimization result factors, namely heating temperature, contact time between fraction rich crude fish oil and activated charcoal, and centrifugation time, in the bleaching purification process given by the Design Expert program have provided optimal results because color data L, a* and b* fall into the prediction range given by the program.

After obtaining the optimization results for the three fraction rich crude fish oil refining processes, the verification stage of these processes was carried out. The parameters of fraction rich crude fish oil refining verification analysis, used as a comparison of fraction rich crude fish oil before refining and after refining, are acid number, peroxide number, iodine number, anisidine number, total oxidation value, etc (show in Tabel 11). The acid number, peroxide number, iodine number, anisidine number, and total oxidation value have met SNI 2018 standards.

Table 11. Results of Verification Analysis of Fraction Rich of Crude Fish Oil Refining

No.	Analysis Parameters	Results	(SNI, 2018)	Description
1	Acid number (mg KOH/g)	1.87 ± 0.02	< 3	Suitable
2	Peroxide number (mEq/Kg)	1.04 ± 0.01	< 5	Suitable
3	Iodine number (g iod/100g oil)	3.16 ± 0.01	> 1,89	Suitable
4	Anisidine number (mgEq/Kg)	11.11 ± 0.01	< 20	Suitable
5	Total oxidation value (mEq/Kg)	13.21 ± 0.01	< 26	Suitable
7	Free fatty acid (%)	8.25 ± 0.01	-	-
8	Saponification numbers (mg KOH/g fat)	2.62 ± 0.12	-	-
9	Total acid (%)	3.25 ± 0.11	-	-
10	pH	6.84 ± 0.23	-	-
11	Total Dissolved Solids (%Brix)	4.625 ± 0.21	-	-
12	Water content (%)	6.052 ± 0.02	-	-
13	Ash content (%)	3.22 ± 0.21	-	-
14	Fat content (%)	4.89± 0.45	-	-
15	Carbohydrate content (%)	57,32± 0.22		
16	Protein content (%)	24.25± 0.34	-	-
17	TPC (CFU/mL)	2.15×10^1	-	-
18	Omega 9 (%)	23.52	-	-
19	Omega 6 (%)	60.49	-	-
20	Omega 3 (%)	16.52	-	-
21	Color: - L - a* - b*	 48.22 ± 0.01 -0.28 ± 0.01 4.41± 0.01	-	-

No.	Analysis Parameters	Results	(SNI, 2018)	Description
22	Refining factor	0.781	-	-

The analysis of fraction rich crude fish oil purification verification shows that the purified fraction rich crude fish oil has met the SNI standards in parameters acid number, peroxide number, iodine number, anisidine number and toral oxidation value. Other analytical parameters serve as characteristics of optimizing the fraction rich crude fish oil refining process. These parameters are free fatty acids, saponification numbers, total acid, pH, total dissolved solids, water content, ash content, fat content, protein content, TPC, omega 9, omega 6, omega 3, and color. The color of good fish oil is green toward yellow and clearer, so L is high, a*, and b*.

Table 12. Percentage Reduction in Fraction Rich Crude Fish Oil Oxidation Parameters

Analysis Parameters	Before Refining	After Refining	Average Reduction (%)
Free Fatty Acids (%)	24.54 ± 0.02	8.25 ± 0.01	66
Acid Number (mg KOH/g)	18.29 ± 0.05	1.87 ± 0.02	90
Peroxide Number (mEq/Kg)	3.93 ± 0.01	1.04 ± 0.01	73.5
Anisidine Number (mgEq/Kg)	28.21 ± 0.01	11.11 ± 0.01	61
Total Oxidation Value (mEq/Kg)	36.07 ± 0.02	13.21 ± 0.01	63
Water Content (%)	76.80 ± 0.12	6.052 ± 0.02	92

Tabel 12 shows the optimal conditions for refining fraction rich crude fish oil with 10% (v/v) NaCl solution, 50°C heating temperature for 10 minutes, and centrifugation for 5 minutes (10,062 G) in degumming refining; with 18°Be NaOH solution, heating temperature 50°C for 20 minutes, and centrifugation for 5 minutes (10,062 G) in neutralization purification; with activated charcoal (b/v 9.4%), heating temperature 80°C for 10 minutes, and centrifugation for 15 minutes (10,062 G) in bleaching purification. These results indicate a reduction in free fatty acids, acid number, peroxide value, anisidine value, total oxidation value, and water content by 66%, 90%, 73.5%, 61%, 63%, 92% respectively.

CONCLUSION

Our research shows that fraction rich crude fish oil can be refined by process of degumming, neutralization and beaching. We identified optimal conditions for each process, as the following: degumming rocess at 50°C for 10 minutes with 5 minutes centrifugation; neutralization at 50°C for 20 minutes with 5 minutes of centrifugation; and bleaching at 80°C for 10 minutes with 15 minutes of centrifugation. Verification of these optimum conditions resulted in refined fraction with significantly improved quality parameters, with free fatty acid value of 8.25% ± 0.01%, acid value of 1.87 ± 0.02 mg KOH/g oil, peroxide value of 1.04 ± 0.01 meq/Kg, anisidine value of 11.11 ± 0.01 meq/Kg, and total oxidation value of 13.21 ± 0.01 meq/Kg. These results indicate a reduction in free fatty acids, acid number, peroxide value, anisidine value, total oxidation value, and water content by 66%, 90%, 73.5%, 61%, 63%, 92% respectively. Optimal conditions produce fraction rich crude fish oil quality parameter response values that comply with the fish oil standard in 2018 in parameter acid number, peroxide number, iodine number, anisidine number, and total oxidation value. While the purification process improved the quality of fraction rich crude fish oil, further processing still is necessary to reduce water content and free fatty acid levels. It's worth noting that the final product still contains carbohydrates and protein. To produce pure fish oil, an advanced process is required, but the resulting product can serve as a food ingredient that is rich in carbohydrates, protein, and fat.

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