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FULL PAPER

Optimization of tilapia (*Oreochromis niloticus*) viscera oil extraction using response surface methodology

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Abstract

Tilapia is one of the export commodities with superior fish fillet products. Fillet processing produces much waste, one of which is viscera, which has the potential as raw material for fish oil. Several factors, including the method, temperature, and extraction time, influenced the quality of fish oil. This study aimed to determine the effect of wet rendering, extraction time and temperature on tilapia viscera oil and determine the best temperature and time based on the characteristics of fish oil using response surface methodology (RSM). The data analysis comprised the peroxide value, free fatty acid, anisidine value, total oxidation, and yield value. The result was then optimized using the response surface method with Design Expert 11. The recommended model is linear. The results for extraction at 60°C for 25.95 mins had a yield, peroxide, free fatty acid, p-anisidine, and total oxidation value of 21.6%, 6.69 meq/kg, 4.16%, 3.87 meq/kg, 17.79, respectively, with, a desirability value of 0.62.

1. Introduction

One of the most dominant freshwater fish produced in Indonesia is tilapia. It is one of the primary products of Indonesian fishery exports. The export product of tilapia is divided into four: whole fresh, whole frozen, fresh fillets, and frozen fillets (Matlata *et al*., 2013; Bilbao-Sainz *et al*., 2020). The high production of tilapia can lead to increased waste from processing the fish. According to Ghaly *et al*. (2013), about 20-80% of the weight of the whole tilapia produced a waste consisting of head, skin, entrails, bones, scale, and trimming results. It would cause environmental problems if the waste were not appropriately handled.

On the other hand, fish waste could be processed as added value because of its high fat and protein content. According to Hossain and Alam (2015), the stomach contents of fish generally consist of the digestive tract, liver, and bile, wherein these organs contain high-fat content. The viscera contained 14.01% protein, 20% lipid, 4.75% ash content and 60.62% water content. The high-fat content in the viscera can be utilized by processing it into fish oil.

Crude fish oil, which is oil that still contains various substances that are consumed, must pass a purification stage (Anandganesh *et al*., 2016). The quality of crude fish oil dramatically influences the quality of fish oil. According to Fuadi *et al*. (2014), good crude fish oil that can be used further must meet the quality standards of IFOMA (International Fishmeal and Oil Manufacturers Association). The standard set by IFOMA fish oil is said to have good quality if it has a free fatty acid content of 1 -7% and a peroxide value of 3-20 meq/kg.

The extraction process influences the quality of crude fish oil. One of the extraction methods that is often done is the rendering method. Rendering is an oil processing process to separate oil, water and solids. Fish oil obtained from this process can be characterized by its physical, chemical and organoleptic properties. According to Eka *et al*. (2016), there are two rendering processes: wet and dry. The principle of extraction with wet rendering is boiling and pressing using water. In contrast, dry rendering extraction does not use water to release the oil, instead removing water from the material.

The rendering must use the right temperature and time to produce good quality fish oil. According to Kamini *et al*. (2016), high temperatures and improper extraction time will trigger the formation of more free radicals and secondary oxidation due to a decomposition process that can break the hydroperoxide component. Optimal temperature treatment and extraction time are crucial to obtain fish oil quality according to IFOS consumption standards.

Putri *et al*. (2020) reported that the extraction of red

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snapper head fish oil using a temperature of 80°C for 60 mins by wet rendering resulted in the highest yield of 9.02%. On the other hand, research by Nurjanah *et al*. (2014), reported that the best extraction of catfish skin oil for 30 mins of extraction at 60°C had 14.37% yield, 60 meq/kg peroxide, 237 meq/kg p-anisidine, 0.8% free fatty acid and 357 meq/kg total, respectively. Research on fish oil extraction was also reported by Martins *et al*. (2021), who extracted catfish oil at a temperature of 100°C for 40 mins to produce the best quality oil, in which the yield was 8.57%, peroxide value of 7.26 meq/kg and 2% free fatty acids. Based on the research mentioned above, it can be seen that the treatment of temperature and extraction time significantly affect the quality of the fish oil produced, so an optimization process of fish oil extraction is needed.

The purpose of this study was to determine the effect of extraction time and temperature wet rendering on tilapia fish oil and to determine the best temperature and time based on the characteristics of fish oil using the RSM (response surface methodology) on yield, peroxide value, free fatty acids, anisidine value, and total oxidation.

2. Materials and methods

2.1 Materials

The primary material used in this research is tilapia viscera from PT. Aquafarm, Semarang, Indonesia and aquades from CV. Indrasari, Semarang, Indonesia.

2.2 Tilapia fish oil extraction

The fish oil extraction method was based on the method described by Putri *et al*. (2020) with some modification. A 500 g Tilapia viscera was added with distilled water (1:1). The extracting process was done according to Central Composite Design (CCD) on RSM software. The research method used was experimental laboratories with a Completely Randomized Design (CRD) data analysis. The research was conducted with 13 treatments and three replications (Table 1).

Table 1. Table model CCD design.

Run	Temperature (°C)	Time (min)
1	55.86	30
2	70	15.86
3	70	30
4	80	20
5	70	30
6	60	20
7	70	30
8	80	40
9	70	30
10	60	40
11	70	30
12	84.14	30
13	70	44.14

2.3 Yield test

The yield of fish oil (%) states the ratio of the weight of fish oil produced (g) to the weight of the sample used (g) (AOAC, 1995). The calculation of the yield of fish oil is as follows:

$$\text{Yield} = \frac{\text{weight of final fish oil}}{\text{initials samples}} \times 100\%$$

2.4 Peroxide value test

A sample of 15 g of fish oil was added to a 250 mL Erlenmeyer flask and 30 mL of a solution of glacial acetic acid and chloroform in the ratio of 3:2. Next, 0.5 mL of saturated potassium iodide solution was added, followed by 30 mL distilled water. The sample was added with 0.5 mL of starch indicator and titrated with $Na_2S_2O_3$ (sodium thiosulfate) until the solution was clear (AOAC, 2005). The formula for calculation of the value of the peroxide number:

$$\text{Peroxide number (meq/kg)} = \frac{\text{A} \times \text{N} \times 100}{\text{weight sample}}$$

2.5 Free fatty acid test

The sample (1.5 g) was put into a 250 mL Erlenmeyer flask, then added with 25 mL of 95% ethanol and heated for 10 mins. The sample was added with two drops of PP indicator and shaken until homogeneous. The titration was conducted with 0.1 N KOH until a pink color appeared and did not disappear for 10 s (AOAC, 2005). The following formula was used for the calculation of the percentage value of free fatty acids:

$$\text{Free fatty acids} = \frac{\text{mL KOH} \times \text{M KOH} \times 56.1}{\text{sample mass (g)}}$$

2.6 p-anisidine test

A sample of 1 mL was put into a measuring flask, then 25 mL of isooctane was added and shaken (A1). The absorbance value was measured using a spectrophotometer with a wavelength of 350 nm. Approximately 5 mL of A1 solution was taken and put into a test tube wrapped with aluminum foil, and 1 mL of p-anisidine was added to acetic acid. The tube was closed, shaken, and left in the dark for 10 mins, which was absorbed in a wavelength of 350 nm (A2) (IUPAC, 1987). The formula calculated the p-anisidine value:

$$\text{p} - \text{anisidine value (p} - \text{AV)} = \frac{25 \times (1.2\text{A2 x} - \text{A1})}{\text{G}}$$

2.7 Total oxidation test

The totox value was added twice the peroxide value to the p-anisidine value (AOCS, 1998). The calculation formula is as follows:

$$\text{Total oxidation value (TOTOX)} = 2\text{PV} + \text{p} - \text{AV}$$

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2.8 Optimization of response surface methodology

The optimization process was conducted with RSM using CCD. The software used is Design Expert 11. The sample extraction process was carried out according to the combination variation of CCD determined by RSM with two factors: the temperature and extraction time.

2.9 Data analysis

Data analysis carried out on the tilapia viscera oil test consisted of yield value, peroxide number (PV), free fatty acid (FFA), p-anisidine value, and total oxidation (TOTOX). The data results were optimized with the response surface method and centralized composite design or CCD using Design Expert 11 software. The optimal solution was verified according to the optimal treatment results from the predicted response surface and the yield value, peroxide number (PV), free fatty acids (FFA), p-anisidine, and total oxidation (TOTOX). The predicted value of the response surface was compared with the actual value.

3. Results and discussion

3.1 Yield

The results of the fish oil yield analysis are shown in Table 2. The lowest yield value obtained at 70℃ temperature treatment in 15.85 mins was 15.6%, while the highest yield at 80℃ temperature treatment in 40 mins was 33.93%. According to Suseno *et al*. (2013), the wet rendering method produced around 1.49-6.44% of tilapia fish oil. According to Nazir *et al*. (2017), different extraction processes produce different yield amounts. The wet rendering extraction produced the highest yield of 12.8% compared to solvent and acid silage. It was shown that this study produced more yield values than previous studies.

The temperature and time increase in the extraction process will also increase the percentage of oil yield obtained (Suseno *et al*., 2013; Suseno *et al*., 2021). Carvajal *et al*. (2014) reported that the protein in the raw material would be coagulated due to the high temperature. The fat would be trapped in the cell tissue at lower temperatures, yielding less. According to Estiasih *et al*. (2021), the extraction time of fish oil is proportional to the yield caused by damage to viscera tissue.

ANOVA test was carried out to determine the model's ability to predict the response, the relationship between factors and responses, and the model's inaccuracy in describing the data. Based on Table 3 it showed that the model is significant because the P-value is less than 0.05, which is <0.001. The f value of the temperature factor and the length of time for extraction were respectively 0.000<0.05; 0.001<0.05. It means that the temperature and length of time the extraction process significantly affect the yield. The P-value on the lack of fit is 0.45 >0.05, which is insignificant. The lack of fit indicates that the model is good. Based on the data above, the selected model is linear.

The regression equation for the linear yield response model, which is influenced by temperature (A) and extraction time (B), is as follows: Yield = +24.38 + 2.87*A + 4.82*B. Figure 1. shows that the linear model is a straight-up curve. The yield value in blue showed the minimum value, while the value in orange is the maximum value. The graph showed the maximum yield value in the treatment using a temperature of 80℃ for 40 mins, which resulted in the highest yield of 33.93%. It concluded that both temperature and extraction time significantly affect the yield value.

Table 2. Result response

No	Variable		Response				
	Temperature (°C)	Time (min)	Yield (%)	PV (meq / kg)	FFA (%)	P-Anisidine (meq/kg)	TOTOX (meq/kg)
1	55.85	30	21	5.97	4.17	3.61	15.55
2	70	15.85	15.6	6.33	4.8	2.68	15.34
3	70	30	24	6.40	5.33	5.50	18.31
4	80	20	24.4	7.11	6.69	5.16	19.38
5	70	30	22.53	8.97	5.65	5.60	23.55
6	60	20	17.66	4.98	4.84	3.31	13.27
7	70	30	22.33	8.33	6.59	5.03	21.70
8	80	40	33.93	10.06	7.15	8.52	28.64
9	70	30	26.66	8.15	6.62	4.89	21.20
10	60	40	27.8	9.09	5.10	5.79	23.98
11	70	30	24	6.87	6.63	5.39	19.15
12	84.14	30	28.13	9.74	7.65	5.83	25.32
13	70	44.14	28.93	10.03	7.30	6.31	26.38

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3.2 Peroxide value

The peroxide value test referred to the oil's hydroperoxide content as the primary oxidation product. The extraction treatment with a temperature of 60℃ for 20 mins produced the lowest PV value was 4.98 meq/kg. The highest PV value obtained at a temperature treatment of 80℃ for 40 mins was 10.06 meq/kg. The high peroxide value in fish oil is caused by the high temperature and time used for extraction. The use of high temperatures causes the formation of primary oxidizing components such as peroxides due to the presence of oxygen in the oil's unsaturated fatty acids. Furthermore, heat treatment will cause the primary oxidation components into lower molecular weight components. This situation will reduce the content of unsaturated fatty acids in fish oil (Ayala *et al*., 2014; Barbosa *et al*., 2019; Rodríguez *et al*., 2021).

Table 3 shows that the model is significant. The respective p-value at each temperature and duration was <0.05, 0.0002 and 0.003. The P-value on the lack of fit in the Table is 0.93, which means it is higher than 0.05, hence insignificant. Therefore, the model is good because the extraction process's temperature and time significantly affect the peroxide number's response. The regression equation for the linear model of the peroxide number response, which is influenced by temperature (A) and extraction time (B) as follows: Peroxide Number = +7.85+1.05*A+1.54*B.

Figure 1 shows a 3-dimensional graph of a linear model with a factor of temperature and fish oil extraction time on the peroxide value response. The graph is in the form of a straight plane because the model is linear. Based on the picture, it was found that a temperature of 60℃ for 20 mins resulted in a response of a minimum peroxide value of 4.98 meq/kg. The extraction treatment's maximum response of 10.06 was obtained using a temperature of 80℃ in 40 mins. The results of the peroxide number in this study were lower than the research of Pudtikajorn *et al*. (2020), who reported that the peroxide number in skipjack tuna oil was 9-18 meq/kg. According to IFOMA (1998), the standard set for crude fish oil to have good quality if it has a peroxide value of 3-20 meq/kg. It concluded that crude fish oil in this study meets IFOMA standards, from the minimum to the maximum response.

3.3 Free fatty acids

Free fatty acids are non-bound fatty acids as triglycerides. These are the result of the hydrolysis and oxidation process. The high percentage of free fatty acids in the oil can have an unpleasant taste and aroma. The lowest free fatty acid value of 4.17% was found in extraction using a temperature of 55.85℃ for 30 mins. Meanwhile, the temperature treatment of 84.14℃ for 30 mins produced the highest free fatty acids at 7.65%. It showed that the higher the temperature and time at the extraction time, the value of free fatty acids will increase. The higher the free fatty acids, the lower the quality of the fish oil. The high value of free fatty acids due to the high temperature caused the triacylglycerol of the oil to be broken so that free fatty acids were formed quickly. It is also supported by the presence of water in the oil, which facilitates the hydrolysis reaction that causes the formation of free fatty acids (Brühl, 2014;

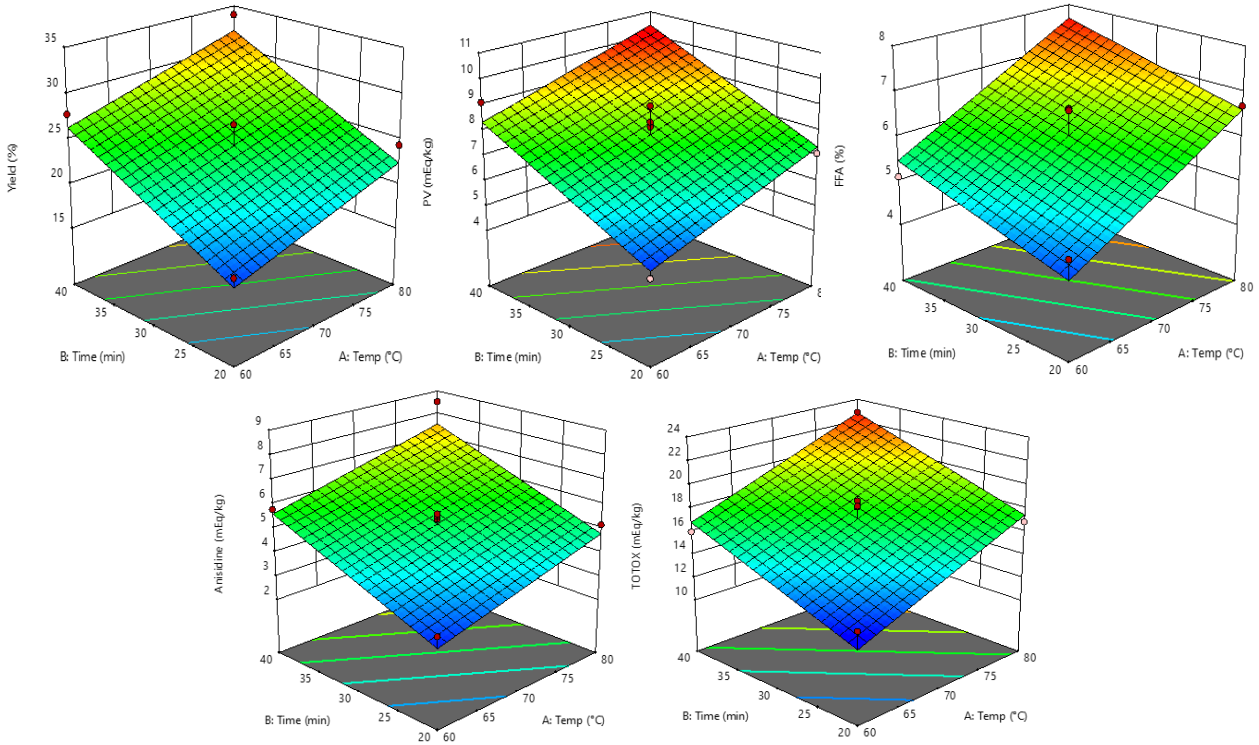

Figure 1. 3D response graph

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Phung *et al*., 2020).

Table 3 shows that the model is significant. It can be seen from both p-values at temperature and time are less than 0.05. The P-value at the temperature is 0.0002, while at the time, 0.01. The temperature and extraction time factors significantly affect the free fatty acid response. It showed that the p-value on the lack of fit has a value of more than 0.05, which is 0.75, which means the lack of fit is insignificant. Lack of fit, which is not significant, indicated that the model is appropriate.

The regression equation for the linear model of the free fatty acid response is influenced by temperature (A) and the length of time (B) extraction as follows: Free fatty acid = +6.04+1.10*A+0.53*B. Figure 1 shows that the extraction temperature factor of 55.85℃ for 30 mins produced a minimum fatty acid response of 4.17%. In comparison, the maximum response of 7.65% was obtained from a temperature treatment of 84.14℃ for 30 mins. According to IFOMA (1998), the good free fatty acids in crude fish oil are 1-7%. Based on these values, the treatment with a temperature of 55.85℃ for 30 mins still meets the IFOMA standard because the free fatty acid value is still in the standard range, but the free fatty acid value of more than seven does not meet the standard. The results in this study were smaller than in the Huli *et al*. (2014) research, who reported that the free fatty acids obtained at 60℃ were equal to 6.92%. In addition, Rozi *et al*. (2014) reported that the value of the lowest fatty acids obtained from the treatment temperature of 50℃ is equal to 5.47%, and the highest value obtained from the treatment temperature of 80℃ was 10.63%.

3.4 p-anisidine

The p-anisidine test to measure the secondary oxidation present in fish oil. The degradation of hydroperoxide compounds will cause the formation of aldehyde and ketone compounds, which are products of secondary oxidation (Eap, 2016; Domínguez *et al*., 2019). The Table 2 showed that the lowest p-anisidine value was found at a temperature of 70℃ for 15.8 mins, which was 2.68 meq/kg. Anisidin p-value of 8.52 meq/kg was on the treatment temperature of 80℃ for 40 mins. P-anisidine is affected by temperature and extraction time. It proves that the lower the temperature, the lower the p-anisidine value. The research of Kamini *et al*. (2016) reported that the anisidine value would be higher if the temperature and extraction time were also higher.

Table 3 shows the analysis used to determine the model's success in predicting the response, the relationship between factors and responses, and the model's inaccuracy. The ANOVA Table of p-anisidine response showed that the model is significant. It can be seen in the p-value listed that the temperature and time were less than 0.05. The p-value at temperature was 0.0008, while at the time was 0.0001. It concluded that the temperature and time factors significantly affect the p -anisidine response. The p-value on *lack of fit* has a value of 0.06, which is said to be insignificant. The insignificant indicated that the model is appropriate.

The regression equation for the linear model of p-anisidine response, which is influenced by temperature (A) and length of time (B) extraction are as follows: p-anisidine = +5.21+0.96*A+1.37*B. Figure 1 shows the minimum response of 2.68 meq/kg was obtained from a temperature treatment of 70^{o}C with an extraction time of 15.8 mins, while a temperature treatment of 80℃ for 40 mins was 8.52 meq/kg. According to Rozi *et al*. (2014), good quality pure fish oil must have a p-anisidine value below 20 meq/kg, while the crude fish oil quality standard based on IFOMA is 4-60 meq/kg. Hence, the results of p-anisidine in this study met the crude oil quality standards according to the IFOMA. The p-anisidine value in this study was smaller than Nurjanah *et al*. (2014), who reported that the lowest anisidine value of 90 meq/kg was obtained at a temperature treatment of 60℃ for 10 mins.

3.5 Total oxidation

The total oxidation value is carried out to estimate the oxidative damage of lipids in fish oil. It was obtained by adding twice the peroxide number with the p-anisidine value. Based on the data, at 60℃ extraction temperature for 20 mins, the total oxidation was 13.22 meq/kg. The highest total oxidation was at the temperature of 80℃ or 40 mins, which was 28.64 meq/kg. It assumed that the total oxidation was affected by temperature and extraction time. The result was lower than the Sardinella fish's total oxidation value, which, extracted using wet rendering, ranged from 35.91–73.67 meq/kg (Suseno *et al*., 2014).

Table 3 shows that the model is significant as the total oxidation response, the p-value listed at a temperature and time less than 0.05. The p-value at temperature was 0.0002, while at the time <0.0001. It can be concluded that the temperature and time factors have a significant effect on the total oxidation response. The p-value on lack of fit has a value of 0.95, which is more than 0.5. The lack of fit is said to be insignificant. The model can be said to be good if it has an insignificant lack of fit.

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Table 3. ANOVA results response.

Parameters	Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Yield	Model	251.46	2	125.73	37.98	<0.0001	Significant
	A-temperature	65.9	1	65.9	19.91	0.001	
	B-time	185.56	1	185.56	56.06	<0.0001	
	Residual	33.1	10	3.31			
	Lack of Fit	21.11	6	3.52	1.17	0.45	Not Significant
	Pure Error	11.99	4	3			
	Cor Total	284.56	12				
Peroxide value	Model	27.82	2	13.91	22.31	0.0002	Significant
	A-temperature	8.9	1	8.9	14.27	0.003	
	B-time	18.93	1	18.93	30.36	0.0003	
	Residual	6.24	10	0.62			
	Lack of Fit	1.67	6	0.27	0.24	0.93	Not Significant
	Pure Error	4.57	4	1, 14			
	Cor Total	34.06	12				
Free fatty acid	Model	12	2	6	21.13	0.0003	Significant
	A-temperature	9.72	1	9.72	34.25	0.0002	
	B-time	2.27	1	2.27	8.01	0.01	
	Residual	2.84	10	0.28			
	Lack of Fit	1.28	6	0.21	0.54	0.75	Not Significant
	Pure Error	1.56	4	0.38			
	Cor Total	14.84	12				
p-anisidine	Model	22.49	2	11.25	33.48	<0.0001	Significant
	A-temperature	7.43	1	7.43	22.12	0.0008	
	B-time	15.06	1	15.06	44.83	<0.0001	
	Residual	3.36	10	0.33			
	Lack of Fit	2.98	6	0.49	5.26	0.06	Not Significant
	Pure Error	0.37	4	0.09			
	Cor Total	25.85	12				
Total oxi-dation	Model	233.85	2	116.92	51.47	<0.0001	Significant
	A-temperature	75.54	1	75.54	33.25	0.0002	
	B-time	158.31	1	158.31	69.69	<0.0001	
	Residual	22.72	10	2.27			
	Lack of Fit	5.28	6	0.88	0.2	0.95	Not Significant
	Pure Error	17.44	4	4.36			
	Cor Total	256.57	12				

The regression equation for the linear model of total oxidation response influenced by temperature (A) and extraction time (B) as follows: Total oxidation = +20.91+3.07*A+4.45*B. Figure 1 is in the form of a straight plane facing up because it has a linear model. It was discovered that the extraction temperature factor of 60°C for 20 mins would produce a minimum response of 13.27, while the maximum response was 28.646 obtained from 80°C extraction temperature for 40 mins. Jairoun *et al*. (2020) reported that higher total oxidation happened because the total oxidation number is the sum of twice the peroxide number, with p-anisidine value as the primary oxidation value (free fatty acid and peroxide number) and the secondary oxidation value (p-anisidine). Based on IFOMA (1998), a good crude fish oil quality standard has a total oxidation value of 10-60 meq/kg, hence, this research met the IFOMA standard. This study has a smaller oxidation value than Rozi *et al*. (2014), with a temperature of 50°C produced a total oxidation value of 31.31 meq/kg, while a temperature of 80°C has 65.29 meq/kg. However, it was higher than Kamini *et al*. (2016), who reported that at a temperature treatment of 50°C for 2 hrs, the total oxidation was 8.38 meq/kg.

3.6 Response optimization

The selected yield response was the maximum target for the highest yield of the various treatments. The peroxides value, free fatty acids, p-anisidine value, and total oxidation were chosen with a minimum target because it is expected that the product would meet the

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quality standards in such a small amount. Based on the criteria, the Design Expert 11 program provides a solution close to the target, as shown in Table 4.

Table 4. Time optimization limit and response.

Name	Goal	Lower Limit	Upper Limit	Importance
A: temperature	is in range	60	80	3
B: time	is in range	20	40	3
Yield	Maximize	15.6	33.93	3
Peroxide value	Minimize	4.98	10.06	3
Free Fatty Acid	Minimize	4.17	7.65	3
p-Anisidine	Minimize	2.68	8.52	3
Total Oxidation	Minimize	13.27	28.64	3

The optimum result predicted by the design expert was the fish oil with a temperature of 60℃ for 25.957 mins produced a yield of 19.567%, 6.176 meq/kg peroxide, 4.724% free fatty acids, 3.687 meq/kg p-anisidine, and 16.040 meq/kg total oxidation as shown in Table 5. The desirability value of the solution is 0.624. According to John (2013), a desirability value close to 1 indicates that the program's ability to produce the desired product is perfect. It means that the response from the results obtained is on target.

3.7 Verification of optimum conditions for prediction results model

Verification is checking the difference between the prediction results provided by the software and the analysis at the optimum point. This verification aims to confirm the actual optimal conditions of the yield response, peroxide value, free fatty acid, p-anisidine, and total oxidation value. The optimal condition for fish crude oil contents using wet rendering was obtained at a temperature of 60℃ for 25.957 mins, compared with the actual validation results with the predictions from the program presented in Table 6.

Based on Table 6, it showed that the yield response results were higher than the predicted value, which was 21.6%. The peroxide value was 6.96 meq/kg, which means it was higher than the predicted result. The free fatty acid value was 2.62%, which was smaller than the predicted value of 4.72%. The p-anisidine value was 3.87 meq/kg, higher than the predicted value. The total oxidation was 17.79 meq/kg, higher than the predicted value. The results mentioned above are between the lowest and highest estimates, so the optimal solution recommended by Design Expert 11 software is considered good.

4. Conclusion

The results of the analysis on the Design Expert 11 software showed that the temperature and the length of the wet rendering method for crude oil from tilapia's viscera significantly affected the yield response, peroxide value, free fatty acid value, p-anisidine, and total oxidation value. The optimization using Design Expert 11 software with RSM-CCD resulted in an optimal process formula with an extraction temperature of 60°C for 25.95 mins.

Conflict of interest

The authors declare no conflict of interest

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Table 5. Solutions produced in the optimization stage.

Temperature	Time	Yield	PV	Free fatty acid	p-anisdine	Total oxidation	Desirability
60.00	25.95	19.56	6.17	4.72	3.68	16.04	0.62

Table 6. Comparison of the predicted validation program with actual results.

Response	Prediction	Verification	low 95% CI	95% CI high	95% PI low	95% PI high
Yield of	19.56	21.6	17.65	21.47	10.54	28.58
Peroxide values	6.17	6.96	5.34	7.00	2.26	10.09
Free Fatty Acids	4.72	2.62	4.16	5.28	2.08	7.36
p-Anisidine	3.68	3.87	3, 07	4.29	0.81	6.56
Total Oxidation	16.03	17.79	14.45	17.62	8.56	23.51

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