

OPTIMIZATION OF SARDINE (*SARDINELLA* SP.) OIL REFINING USING RESPONSE SURFACE METHOD (RSM)

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ABSTRACT:

RSM (Response Surface Method) is a set of mathematical techniques and statistics that are useful to analyze the problems so that the level of response was influenced by several variables. This research aimed to determine the optimal treatment of sardine (*Sardinella* sp.) oil refining through centrifugation and adsorption. Based on the results, it can be concluded that the optimum treatment was centrifugation at 9494.89 rpm for 31.48 minutes with addition of combined adsorbents attapulgite and bentonite (1:1) 3%. The optimum point resulted a peroxide value at 2.97 meq/Kg, p-anisidine value at 0.81 meq/Kg, totox value at 6.32 meq/kg, and free fatty acid at 4.07%

Keywords— adsorbent, refining, RSM, sardine oil

INTRODUCTION

Optimization techniques using response surface method (RSM) is a systematic approach that enables a number of variables studied simultaneously. Montgomery (2005) stated that RSM is a set of mathematical techniques and statistics that are used full to analyze the problems that the level of response was influenced by several variables, the goal is to optimize the response. Advantages of using the RSM are can make the optimum region and reduce repeated experiments that require a lot of time and costs. Wanasundara and Shahidi (1999) stated that the method is effective for optimizing the process and has been widely used in the research of optimization.

Refining of sardines oil from Pekalongan was analyzed using a complete random design in study of Suseno *et al.*, (*In Press*) showed that the best treatment for obtaining best quality of fish oil was the combination between centrifugation (10,500 rpm for 30 minutes) and addition of synthetic adsorbents (attapulgite: bentonite 3%). Analysis results with a complete random design can be optimized using response surface method (RSM) and thus obtained refining treatment with variable speed, time, and adsorbent in a wider scale. Response surface method (RSM) is required for efficient fish oil refining so the treatment in production process can be improved and developed.

MATERIALS AND METHODS

The sardines oil was obtained from fish meal industry in Pekalongan, Central Java (Indonesia) and adsorbents (attapulgate and bentonite). The materials used for the quality analysis comprised ethanol 96%, phenolphthalein indicator, KOH 0.1N, chloroform, glacial acetic acid, saturated solution of KI, aquades, starch solution 1%, Na₂S₂O₃ 0.1 N, isooctan, anisidine reagent, and n-hexane.

Sampele treatments: Sardines oil was centrifuged using different speed and time (2,500, 4,500, 6,500, 8,500, and 10,500 rpm for 15, 30 and 45 minutes) at 10°C. Fish oil from the best centrifugation treatment was then purified using adsorbents (attapulgate:bentonite 3%) by stirring the mixture of fish oil

and adsorbent for 20 minutes at 29 °C. Adsorbent was removed using centrifugation treatment at 10,000 rpm, at 10 °C for 30 minutes and purified fish oil was analyzed.

Quality analysis

Peroxide value (AOAC, 2000, Method Number 965.33b): Oil sample (2 g) was dissolved in 30 mL chloroform: acetic acid (3:2, v/v) then 1 mL freshly prepared saturated KI (potassium iodide) solution was added and the mixture was vortexed for 1 min. About 30 mL distilled water and 0.5 mL starch solution were added and the liberated iodine was titrated with sodium thiosulfate (0.1 mol L⁻¹). Peroxide value in the unit meq/kg was determined by the following equation:

$$\text{Peroxide value (meq/kg)} = \frac{(S-B) \times N \times 1000}{G}$$

S = mL Na₂S₂O₃ sample; B = mL Na₂S₂O₃ blanco; N = normality Na₂S₂O₃;
G = samples weight

Anisidine value/p-AV (Watson, 1994): Solution 1 was prepared by dissolving 0.5g samples into 25mL trimethylpentane and solution 2 was prepared by adding 1 mL p-anisidine reagent (2.5 g/L) into 5 mL solution 1, then the mixture was shaken in the dark. Reference solution was made by adding 1 mL p-anisidine reagent (2.5

g/L) into 5 mL trimethylpentane, the mixture was then shaken and avoid from the light. Absorbance value of all three solutions made was measured using UV-Vis spectrophotometer using wavelength 350 nm after 10 minutes of solution preparation (Watson, 1994).

The value of anisidine set with an equation the following:

$$\text{Anisidine value} = (25 \times (1,2 A1-A2))/m$$

A1= absorbance solution 1; A2 = absorbance solution 2; m = sample weight used for solution 1

Free fatty acid (FFA) (AOAC, 1995): Oil was weighed into a flask followed

by neutralization using 95% ethanol, after that the mixture was heated using

waterbath for 10 minutes. Phenolphthalein indicator was then added to the heated oil. The mixture was titrated against sodium hydroxide solution until a permanent pink color persisted for at least 30 s. Percentage of FFA by weight was calculated on either an

oleic, palmitic, or lauric acid basis, depending on the type of oil being analyzed (AOAC, 1995). Each sample was titrated in triplicate. FFA percentage was calculated based on the following equation:

$$\text{FFA (\%)} = \frac{A \times N \times M}{10G}$$

A = the number of titration using KOH (mL); N = normality of KOH
G = samples weight; M = Fatty acids dominant molecular weight

Total oxidation/totox (Perrin, 1996):

Total oxidation value was obtained by summing the value 2PV with PAV, where PV is the peroxide value and p-

AV is the p-anisidine (Perrin, 1996). Total oxidation was calculated based on the following equation:

$$\text{Total oxidation} = 2PV + p\text{-AV}$$

Statistical Analysis: Data of fish oil quality as a result of refining was analyzed using response surface method. Central composite design was used in the experiment and data was statistically analyzed through minitab 15 software.

RESULTS AND DISCUSSION

Determination of optimum model:

Optimization with Response Surface Method (RSM) is presented in Table 1. The model selection was based on the extent of the factor being tested that had been approaching the optimum value so that there is an arch (curvature). Second order model was used for creating the experimental design. Experimental design for

second order model was a central composite design. RSM method was used to determine the appropriate model. The models evaluated were linear, interaction, quadratic and cubic model. Chen *et al.*, (2005) stated that the model analysis was used to determine the appropriate model in the response surface methods, with $p < 0.05$ expressed as significant, the highest order polynomial model. Estiasih *et al.*, (2005) stated that the model selected according to the lowest standard deviation value, the lowest PRESS (Prediction Error Sum of Squares) value, and the highest value of R-squared, adjusted R-squared, and predicted R-squared.

Table 1: Central composite design for optimization and its response values (a treatment was centrifugation which then followed by purification using adsorbent)

Code variable		Original variable			Response value		
Centrifugation	Time	Centrifugation speed (rpm)	Time (minutes)	Peroxide value	p-anisidine value	FFA value	Totox value
-1	-1	8,500	20	3.25	0,9	4.58	7.19
0	0	10,500	30	3	0.51	5.29	6.51
0	0	10,500	30	3.25	0.69	4.58	7.19
-1	1	8,500	40	3.25	1.00	3.38	7.50
1	0	13,328.43	30	3	0.51	5.29	6.51
1	-1	12,500	20	3	0.85	4.44	6.85
0	0	10,500	30	3	0.85	4.44	6.85
0	-1.41	10,500	15.86	3.13	0.85	4.56	7.10
0	1.41	10,500	44.14	2.88	0.85	4.32	6.60
0	0	10,500	30	3	0.85	4.44	6.85
-1.41	0	7,671.57	30	4.25	0.69	5.15	9.19
0	0	10,500	30	4.25	0.89	5.36	9.39
1	1	12,500	40	3.25	0.83	5.43	7.33

Note: peroxide value (meq/Kg), FFA (%), p-anisidine value (meq/Kg), totox value (meq/Kg)

Based on statistical modelling calculation which includes a description of the square value and sequence model (Model of Sequential Sum Square) as well as a swift test model (lack of fit), the model selected

for response values of peroxides, p-anisidine, total oxidation and the free fatty acid value was quadratic model. Regression equations for real variables RSM can be seen in Table 2.

Table 2: Regression equations for the RSM real variables

Variable	Equation
Peroxide value	$Y_2 = 1.649644672 + 0.09302208X_1 - 0.03789478X_2 - 0.09130982X_1X_2 + 0.156438264X_1^2 + 0.124145437X_2^2$
P-anisidine value	$Y_3 = 0.825961538 - 0.099723372X_1 - 0.030042839X_2 + 0.130625X_1X_2$
FFA	$Y_4 = 4.170075 + 0.199404112X_1 + 0.385978598X_2 + 0.282X_1X_2 + 0.215025X_1^2 + 0.320775X_2^2$
TOTOX	$Y_6 = 6.3055 - 0.385756403X_1 + 0.374733496X_2 + 0.239125X_1^2 + 1.024125X_2^2$

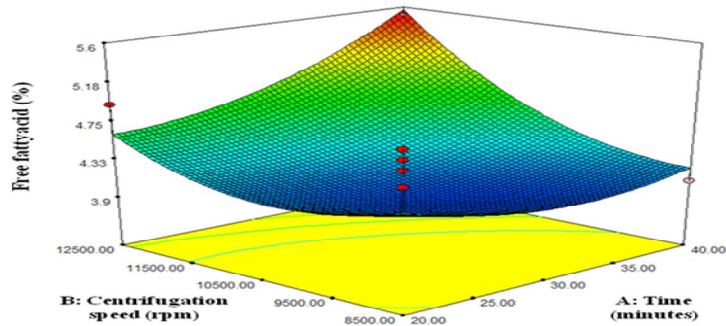
Note: X_1 = centrifugation speed (rpm), X_2 = time (minute)

Response surface and optimum point : Free fatty acid (FFA): Free fatty acids is very concerned with flavor and texture that are less

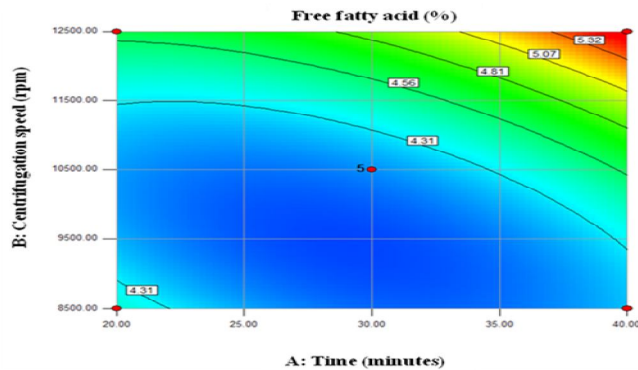
attractive on the oil. The amount of FFA in oil processing industry is very concerned with the requirement of alkali which will be used in the

refining process (Sathivel *et al.*, 2003). FFA is a product of the hydrolysis reaction on triacylglyceride, and closely associated with the storage

process. Optimization model for the value of FFA as a response is presented on Figure 1.



(a)



(b)

Figure 1: RSM Model with FFA as A Response, (a) Three-Dimensional Model, (b) Flat Plot Model

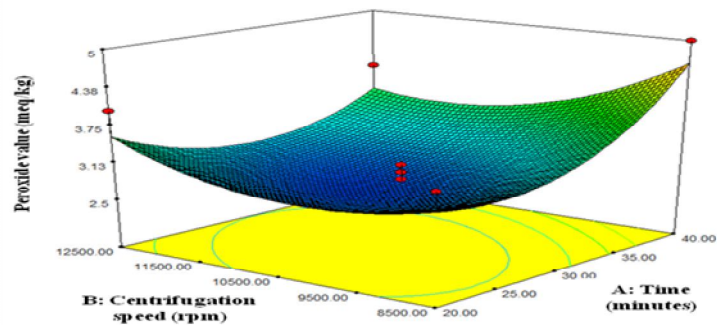
RSM model in Figure 1 shows that the minimum FFA value was reached at a treatment of centrifugation at 9,500-10,500 rpm for 25-30 minute with the addition of adsorbent (attapulgate: bentonite 3%). This result was much still in decent value for fish oil consumption recommended by Bimbo (1998) that is 1-7%. Based on the

results of RSM model, FFA values which were generated by the refining treatment at centrifugation speed 8,500-12,500 rpm for 20-40 minutes were appropriated to the standard which according to Bimbo (1998) but not appropriated to IFOS (2011).

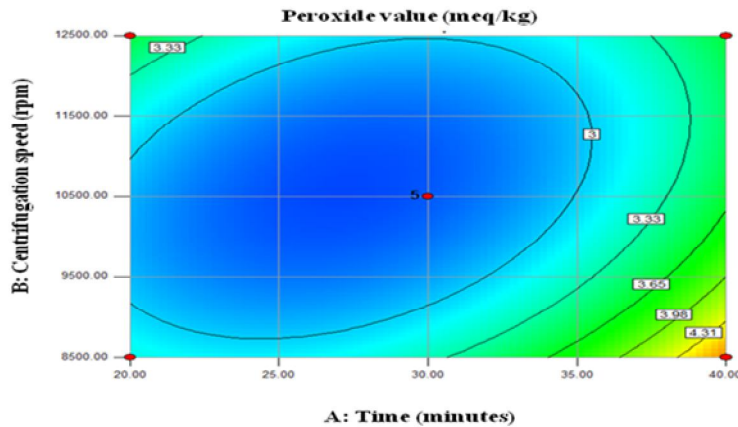
Peroxide Value: Peroxide value analysis aimed to see how much

hydroperoxides as the primary product of oxidation process which were contained in the oil (Aidos *et al.*, 2001). Oxidation value is very important as an indicator of oil quality, the lower value of primary and secondary oxidation product results in the better quality of the oil. Formation of hydroperoxide in oxidation process is generally measured as peroxide value

(Aidos *et al.*, 2003). According to European Commission Regulation (2006), fish oil quality which indicated by some parameters such as free fatty acids, moisture content, color, anisidine value, and peroxide value will determine the price of fish oil in the market. Optimization model for peroxide values as a response is presented in Figure 2.



(a)



(b)

Figure 2: RSM Model with Peroxide Value as A Response, (A) Three-Dimensional Model, (B) Flat Plot Model

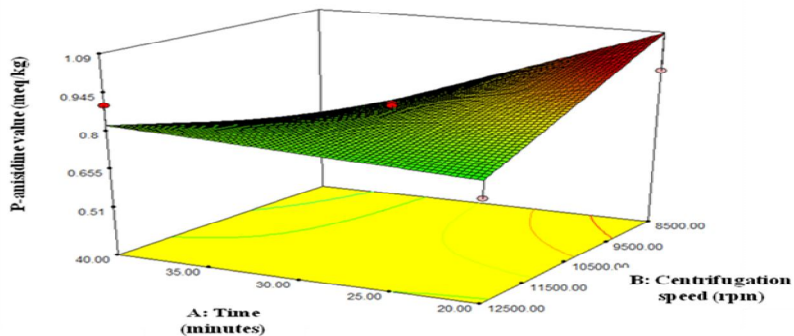
The data in Figure 2 shows that the minimum peroxide value was achieved

at the speed centrifugation 10,500 rpm for 25-30 minutes with the addition of

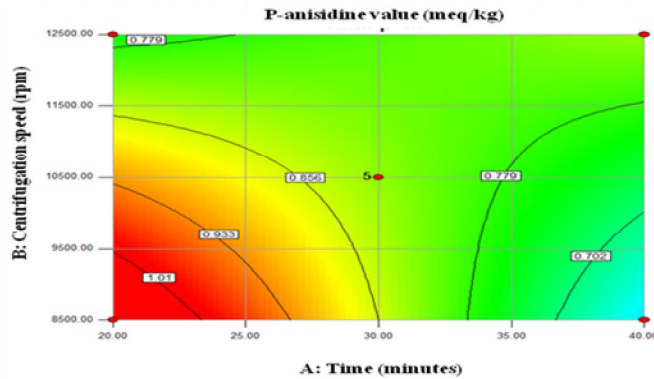
adsorbent (attapulgit:bentonite 3%). The peroxide value was < 2.97 meq/kg. The results obtained were in accordance to decent value of fish oil standard which is recommended by Bimbo (1998), he stated that peroxide value of oil which is fit for consumption ranged from 3-20 meq/kg. The standard for peroxide value which set by BPOM – RI was below 5 meq/kg and according to IFOS (2011), peroxide value must be below 3.75 meq/kg to fit in the category of decent fish oil for consumption. Based on the results of RSM, peroxide values generated by the refining treatment with centrifugation speed at 8,500-12,500 rpm for 20-40 minutes were appropriated to the standard recommended by Bimbo (1998), while the

treatment of centrifugation speed at 8,500-12,500 rpm for 20-35 minutes result the fish oil which fit to IFOS (2011).

P-anisidine Value: P-anisidine value is characterized by secondary oxidation products from fat degradation initiated by hydroperoxide and carbonyl resulting in non-volatile by-products (Aidos *et al.*, 2003). Anisidine value can determine the presence of aldehydes in the oil, because according to O'Brien (2009), aldehydes in the oil and reagents react in acid condition and oil color expression is highly dependent on the amount and structure of the aldehyde. Optimization model for value anisidine as a response is presented in Figure 3.



(a)



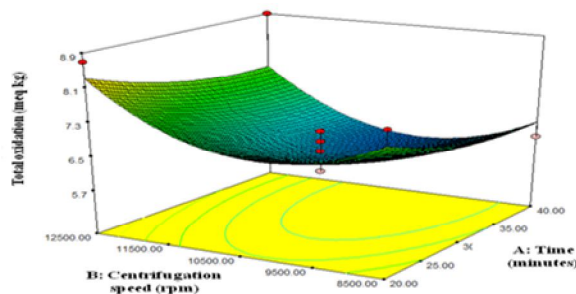
(b)

Figure 3: RSM Model for P-anisidine Value as A Response, (a) Three-Dimensional Model, (b) Flat Plot Model

RSM Model in Figure 3 shows that the minimum value of p-anisidine was obtained at a treatment of centrifugation speed below 9500 rpm and period under 40 minutes with the addition adsorbents (attapulgit: bentonite 3%). P-anisidine value obtained in this study was still in a decent standard fish oil consumption anisidine value ≤ 0.82 meq/Kg. Fish oils fit for consumption must have anisidine value ≤ 20 mEq/Kg (Hamilton *et al.*, 1988), 4-60 meq/Kg (Bimbo, 1998) ≤ 15 meq/Kg (IFOS, 2011). Results of RSM, anisidine

value generated by the refining treatment to speed 8500-12500 rpm for 20-40 minutes, were also included in the standard Bimbo (1998), Hamilton *et al.*, (1988) and IFOS (2011).

Total oxidation (totox) value: Totox value is the relationship of primary and secondary oxidation obtained by summing twice of peroxide value (2PV) and anisidine value (PAV) (Perrin, 1996). Optimization model for the totox value as a response is presented in Figure 4.



(a)

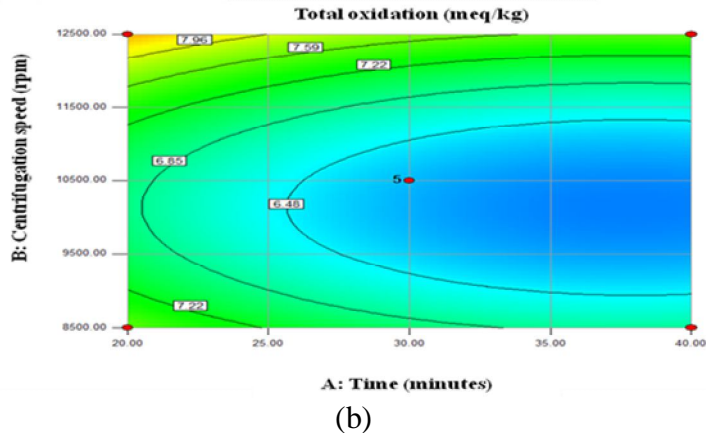


Figure 4: RSM Model for Total Oxidation Value as A Response, (a) Three-Dimensional Model, (b) Flat Plot Model

RSM modeling in Figure 4 shows that the minimum values were reached at a treatment of 9500-10500 rpm as centrifugation speed and 35-40 minutes as time period of centrifugation. After centrifugation, there was the addition of adsorbents (attapulgit:bentonite 3%). Torox values were reached to < 6.27 meq/kg. The results obtained in this study were appropriate to the standard as recommended by Bimbo (1998) stating totox value for a decent oil consumption ranged from 10-60 meq/Kg. While IFOS (2011) stated fish oil for consumption must have a totox value under 20 meq/Kg. Based on the result, totox values generated by the refining treatment with a centrifugation speed of 8500-12500 rpm for 20-40 minutes were

appropriate to the standard as recommended by Bimbo (1998) and IFOS (2011).

Overlay plot: Overlay plot is a technique for combining multiple and variable responses in a single graph, so a conclusion can be drawn based on the interaction of the response variable and the value of each graph. The optimum point which could reach lowest value of primary and secondary oxidation products was achieved in the treatment of centrifugation speed 9494.89 rpm for 31.48 minutes, and then followed by the addition of adsorbents (attapulgit:bentonite 3%) that resulted peroxide value at 2.97 meq/Kg, p-anisidine value at 0.81 meq/Kg, totox value at 6.32 meq/kg, and free fatty acid at 4.07%. The overlay plot can be seen in Figure 5.

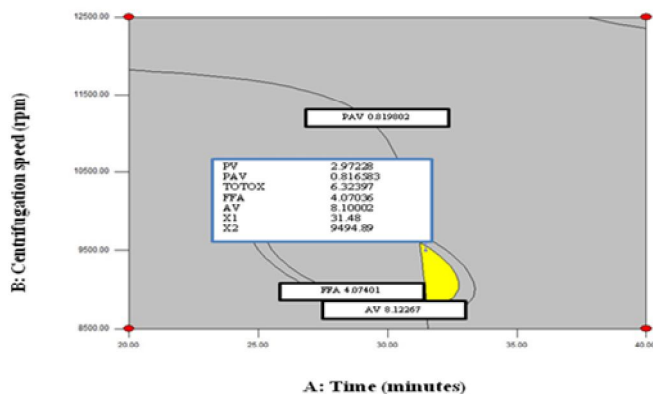


Figure 5: Overlay Plot

CONCLUSION

Based on the results of the optimization of sardine oil refining, it can be concluded that the optimum point was obtained by a treatment of centrifugation at 9494.89 rpm for 31.48 minutes and then followed by the addition of combined adsorbents (attapulgate : bentonite 3%). The optimum point resulted a peroxide value at 2.97 meq/Kg, p-anisidine value at 0.81 meq/Kg, totox value at 6.32 meq/kg, and free fatty acid at 4.07%.

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