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Optimization of Bleaching Condition for Sardine Oil from Fish Meal By-Product

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Abstact: Sardine oil obtained from by-product meal processing industry is an abundant source of ω-3 fatty acids. Utilization of oil from fish meal by-products is very limited, due to its less attractive color and high FFA level. In order to optimalize its utilization, this fish oil needs to be purified. Bleaching technique was conducted in this study. Miracle Filter Powder (MFP) was used as adsorbent. Central composite design of response surface method was employed as experimental design with three variables, i.e. adsorbent concentration, temperature and period toward response of peroxide value (PV) and Free Fatty Acid (FFA). The results showed that quadratic regression model was suitable to explain the interaction of all variables toward response of PV and FFA. Optimum condition was obtained at a treatment of MFP 3% addition, with temperature and contact period were 50°C and 15 min, respectively. Results of validation showed that optimum condition resulted the 21.68% reduction of PV and 30.68% reduction of FFA.

Key words: Bleaching · Free Fatty Acid · Lemuru Oil · Miracle Filter Powder · Optimization · Peroxide Value

INTRODUCTION

Sardine (*Sardinella* sp.) is quite important fishery commodity of the Bali's Strait. Based on statistical data of Ministry of Fisheries and Marine Affairs, sardine's production of Bali Province reached 5573 tons in 2012 [1]. This fish used as raw material in the canned fish and fishmeal industry. Fishmeal processing produced 5% fish oil as by-product [2]. Oil from fishmeal by-products utilization is very limited, due to its less attractive color and its high free fatty acid (FFA) level. Purification of oil from fishmeal by-product is required, so it can give benefit for wide utilization, either for industrial, pharmaceutical and nutraceutical field.

Fish oil purification can remove impurities components and non-triacylglycerol component so it can enhance the acceptability and storability of fish oil [3]. One example of purification technique is adsorption by adsorbent, or so-called bleaching treatment. The adsorbent can adsorb impurities components, pigments and free fatty acids in fish oil.

The adsorbent used in this study was Miracle Filter Powder (MFP). MFP contains magnesium silicate. Magnesium silicate has large active surface area and provides alkalinity in the oil so it has good ability to absorb acids and polar compounds [4]. The study of bleaching process is an important issue to be considered in order to produce fish oil for human consumption.

This study aimed to optimize the operational conditions of the bleaching process for sardine oil using Respond Surface Method (RSM). In this research, the in?uence of adsorbent concentration, temperature and bleaching period on key oxidation (Free fatty acids and peroxide) parameters was evaluated.

MATERIALS AND METHODS

Materials and Equipments: Semi refined oil was used in this work. Semi refined oil is defined as crude sardine oil which has been refined using alkali, so its soapstock might be reduced. It was taken from fish meal industry by-product in Bali, Indonesia and kept in chilling temperature. Miracle Filter Powder was used as adsorbent agent. Other supporting materials were KOH 0.1 N, sodium thiosulfate ($Na_2S_2O_3$) 0.01 N, glacial acetic acid, chloroform, potassium iodide (KI), phenolphthalein indicator (Indicators pp), starch, ethanol 96% and distilled water. Some equipments used were digital scales, burrets, glass tools, stop watch, aluminium foil, water bath and micro pipette.

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Table 1: Central composite design for bleaching operation

Independent variables	Code	Level -1,68	-1	0	1	1,68
Adsorbent concentration (%)	X_1	1,3	2	3	4	4,7
Temperature (°C)	X_2	28	30	50	70	80
Time (minutes)	X_3	7	10	15	20	23

Bleaching Preliminary Experiment: Fish oil purification was conducted in three stages. Firstly, fish oil sample was added by *Miracle Filter Powder* 1, 2, 3, 4 and 5% (w/w). The mixture was stirred by magnetic strirrer at room temperature (28°C) for 20 minutes, then decanted for 5-10 minutes. After obtaining the best adsorbent concentration, adsorption temperature was determined, the temperature treatment used was room temperature (28°C), 50, 70 and 90°C. After obtaining the best adsorption temperature, adsorption period was determined, period treatment was 5, 10, 15 and 20 minutes. The bleaching process was carried out as same as determining the adsorbent concentration process.

Optimization by RSM: Preliminary experiment was conducted to get the range for the experiments. Software package, Design- Expert version 7.0.0 (Statease Inc., Minneapolis, USA) was applied in this optimization study. Central Composite Design (CCD) with three variables and five levels was designed as belowed to study the response pattern and establish a model. Table 1 show the bleaching operation design for these experiment.

Determination of Oxidation Parameters

1. Free Fatty Acids (FFA): The percentage of FFA in each sample was determined by the titration method as described in AOCS method Ca 5a-40 [5]. A total of 2 g of oil was dissolved in 25 ml of 96% neutral alcohol (200 ml erlenmeyer), heated for 10 minutes, then the mixture was then spilled by 2 ml phenolphtalein indicator. The mixture was shaken and titrated with 0.1 N KOH until the pink color arises not lost in 10 seconds. The percentage of FFA was calculated by the following equation:

$$\%FFA = \frac{V \times N \times 282.5}{G}$$

V: Number of KOH titration (ml)
N: Normality of KOH
G: Weight of sample
282.5: Molecular weight of oleic acid

Peroxide Values: The peroxide value (PV) of the oil was carried out according to the AOCS Official Method Cd-8b-90 [5]. About 5 g of sample in 250 ml erlenmeyer was added by 30 ml of acetic acid and chloroform (3:2), then added by 0.5 ml of saturated potassium iodide (KI) solution with stirring and 30 ml of distilled water. Titration was carried out using 0.01 N sodium thisulfate ($Na_2S_2O_3$) until the solution color changed to yellow, then 0.5 ml of 1% starch indicator solution was added until the solution color changed to blue. Titration was carefully continued until the blue color of the solution disappeared.

Peroxide value calculation was obtained with the following equation:

$$PV = \frac{V \times N \times 1000}{G}$$

V: The number of $Na_2S_2O_3$ titration (ml)
N: Normality $Na_2S_2O_3$ (0.01)
G: Weight of sample (g)

RESULTS AND DISCUSSION

Effect of adsorbent concentration, temperature and period of bleaching on the experimental value for peroxide velue (PV) and free fatty acid (FFA) is shown in Table 2.

Analysis of Factor Combination Effect to PV as Response: Results of the analysis found that there are several possible models that can be selected to illustrate the effect of the adsorbent concentration, temperature and period on the parameter of peroxide value and free fatty acids. Some design models are linear, linear with interaction, quadratic and cubic. There were three methods used to select model based on calculations using the program Design Expert 7.0.0, i.e. Sequential Model Sum of Squares (SMSS), lack of fit and a summary of statistics. The summary for determining appropriate model which peroxide value (PV) was set as response can be seenin Table 3.

Table 2: Experimental design and measured values for the response variables

Exp.	Adsorbent concentration (%)	Temp. (°C)	Period (min)	PV (meq/kg)	FFA (%)
1	2	30	10	11.45	1.29
2	4	30	10	10.93	0.95
3	2	70	10	13.07	1.03
4	4	70	10	12.23	1.24
5	2	30	20	13.1	1.26
6	4	30	20	12.33	1.02
7	2	70	20	10.62	1.01
8	4	70	20	10.96	0.88
9	1.3	50	15	13.05	1.3
10	4.7	50	15	12.25	1.19
11	3	28	15	11.86	0.97
12	3	80	15	12.13	1.19
13	3	50	7	12.24	1.24
14	3	50	23	13.17	1.13
15	3	50	15	9.76	0.83
16	3	50	15	11.69	0.98
17	3	50	15	10.2	0.96
18	3	50	15	10.81	1.13
19	3	50	15	8.98	0.91
20	3	50	15	9.54	0.86

Tabel 3: Summary for determining appropriate model (PV as response)

Parameter	*SMSS Prob>F*	*Lack of fit Prob>F*	R^2	*Adjusted* R^2	Annotation
Linear	0.9309	0.1774	0.0266	-0.1559	
2FI	0.3819	0.1687	0.2245	-0.1334	
Quadratic	0.0073	0.7314	0.7549	0.5343	*Suggested*

Tabel 4: Result of ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	Df	Mean Square	F Value	P-value Prob > F	
Model	22.55877	9	2.50653	3.422215	0.0343	Significant
A-Concentration	0.719855	1	0.719855	0.982832	0.3449	
B-Temperature	0.016585	1	0.016585	0.022644	0.8834	
C-Time	0.058532	1	0.058532	0.079914	0.7832	
AB	0.078013	1	0.078013	0.106512	0.7509	
AC	0.108113	1	0.108113	0.147608	0.7089	
BC	5.729113	1	5.729113	7.822073	0.0189	
A^2	7.47875	1	7.47875	10.21089	0.0096	
B^2	3.443293	1	3.443293	4.701197	0.0553	
C^2	7.88795	1	7.88795	10.76958	0.0083	
Residual	7.324289	10	0.732429			
Lack of Fit	2.622556	5	0.524511	0.557785	0.7314	not significant
Pure Error	4.701733	5	0.940347			
Cor Total	29.88306	19				

The analysis showed that the quadratic model has significant value SMSs with the "Prob> F" is smaller than 0.05 (0.0073). Lack of fit values obtained "Prob> F" greater than 0.05 (0.7314) means there is no lack of fit (Not significant). Not significant lack of fit value indicates that the response data (Peroxide value) is compatible with the model. R^2 value of 0.7549 means that the influence of variables X1, X2 and X3 to change the variable response was 75.49% while the remaining 24.51% influenced by other variables that are unknown. Based on three criterias for model selection, the chosen model which can describe the relationship among all variables to response of Y1 (PV) is quadratic model.

Analysis of variance (ANOVA) result can show us the effect of each factor on the response parameter. Linear interaction between bleaching temperature and bleaching period gave a significant effect to response change. Its interaction had p-value "Prob> F" which was less than 0.05 (0.0189). ANOVA result can be seen in Table 4.

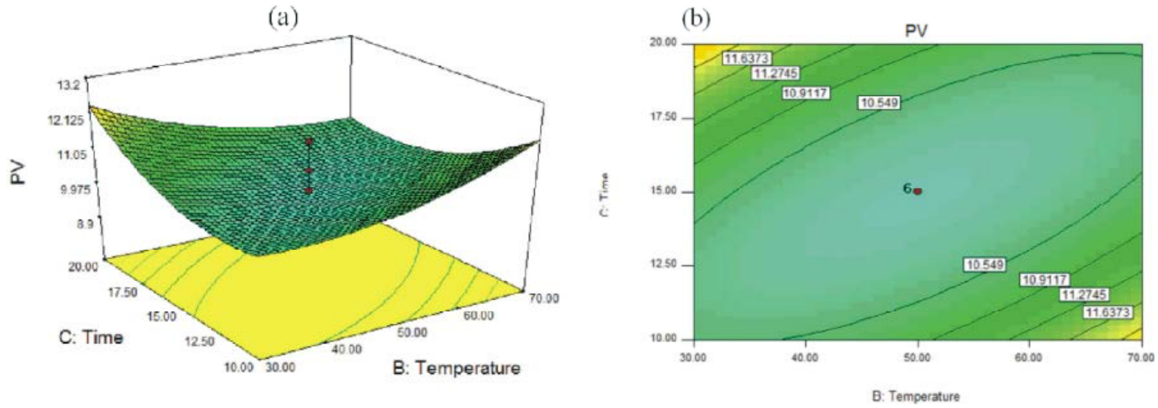

Fig. 1: Effect of temperature and contact period of fish oil bleaching to PV as response: chart (a) 3-Dimensional response and (b) contour

Here is a polynomial equation model forperoxide value as a response:

$$\text{Peroxide value } (Y) = 22.40403 - 5.14751\,X_1 - 0.011819\,X_2 - 0.52133\,X_3 + 4.9375\,X_1X_2 + 0.02325\,X_1X_3 - 8.462\,X_2X_3 + 0.72038\,X_1^2 + 1.22201\,X_2^2 + 0.029593\,X_3^2$$

X_1 = Adsorbent consentration (%)
X_2 = Temperature (°C) X_3 = Period (Minutes)

Based on the above model equation, it can be seen that the peroxide value tend to decrease with the increasing of adsorbent concentrations (X_1), bleaching temperature (X_2) and bleaching period (X_3), as well as the interaction between bleaching temperature and period (X_2X_3).

Based on Figure 1(b), the lowest value of PV can be found in the center of horizontal circular contour lines. Blue area shows the lowest peroxide. Six central point on the contour is right at the center point of the circle. The condition shows that the best response value will be obtained by conditioning factors at a central point.

The tendency of peroxide value as response function in Figure 1 shows that higher temperatures can produce high reduction of PV. The higher temperature, the greater kinetic energy which is used for molecule collision, so the ability of the adsorbent to adsorb the compounds peroxide (PV) will also increase. However, if temperature is too high, it wil give adverse effects to final product, because high temperature can accelerate the formation of peroxide compounds [6].

Besides bleaching temperature and period, the concentration of adsorbent can affect PV reduction too. Too high adsorbent concentration allowing the adsorption of impurities components occurs maximally, but it allows the natural antioxidants contained in pigments absorbed so that it can affect the stability of oxidation of fish oil. According to Dimic *et al.* [7] the bleaching process was particularly considered as undesirable, because large amounts of bleaching earth promote a loss of tocopherols, further affecting the oil stability. Then, List *et al*. [8] also reported that sunflower oils treated in the laboratory with a high concentration of bleaching earth (6%) had poorer flavour and oxidative stability than the same oil bleached with a lower concentration of bleaching earth (2%). Bleaching could remove, destroy, or inactivate some unknown minor constituent which is essential for keeping optimum quality.

Analysis of Factor Combination Effect to FFA as Response: Table 5 shows the summary of statistics analysis for determining appropriate model resulting optimum condition process in reducing free fatty acid (FFA) level.

Based on Table 5, it can be seen that the Sequential Model Sum of Squares is not significant, the value "Prob > F" is more than 0.05 (0.0684), but the lack of fit values obtained "Prob> F" greater than 0.05 (0.2821) means no lack of fit (Not significant). Lack of fit value which is not significant indicates that the response data is compatible with the model of free fatty acids as response. R^2 value is 0.6503 means that the influence of variables X_1, X_2 and X_3 to change the response variable was 65.03% while the remaining 34.97% was influenced by other variables that are unknown. Based on the three criteria for model selection, the chosen model which could describe well about the relationship between all variables and response Y2 (FFA) is a quadratic model.

Table 5: Summary for determining appropriate model (FFA as response)

Parameter	*SMSS Prob>F*	*Lack of fit Prob>F*	R^2	*Adjusted* R^2	Annotation
Linear	0.5427	0.1455	0.1221	-0.0425	
2FI	0.3570	0.1411	0.3094	-0.0094	
Quadratic	0.0684	0.2821	0.6503	0.3355	*Suggested*

Table 6: Result of ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	df	Mean Square	F Value	P-value Prob > F	
Model	0.290572	9	0.032286	2.065859	0.1369	not significant
A-Concentration	0.034358	1	0.034358	2.198447	0.1690	
B-Temperature	7.31E-06	1	7.31E-06	0.000468	0.9832	
C-Time	0.020182	1	0.020182	1.291375	0.2823	
AB	0.05445	1	0.05445	3.48407	0.0915	
AC	0.0072	1	0.0072	0.460704	0.5127	
BC	0.02205	1	0.02205	1.410905	0.2624	
A^2	0.104732	1	0.104732	6.701446	0.0270	
B^2	0.010438	1	0.010438	0.667882	0.4328	
C^2	0.059094	1	0.059094	3.781245	0.0805	
Residual	0.156283	10	0.015628			
Lack of Fit	0.098933	5	0.019787	1.725069	0.2821	not significant
Pure Error	0.05735	5	0.01147			
Cor Total	0.446855	19				

Result of analysis of variance (ANOVA) can show the influence of each factor on the free fatty acids level as response. The quadratic interaction of adsorbent concentration showed the lowest p-value, which was 0.0270. It means that quadratic interaction of adsorbent concentration give significant effect to FFA level. ANOVA result can be seen in Table 6.

The following is polynomial equation model for free fatty acids level as response:

$$\text{Response Free Fatty Acid (Y)} = 2.67892 - 0.6779\,X_1 - 0.011192\,X_2 - 0.0403\,X_3 + 4.125\,X_1X_2 - 6.00\,X_1X_3 - 5.250\,X_2X_3 + 0.085249\,X_1^2 + 6.72812\,X_2^2 + 2.5614\,X_3^2$$

X_1 = Adsorbent consentration (%)
X_2 = Temperature (°C), X_3 = Period (Minutes)

Based on the above equation model, it can be seen that free fatty acid level tend to decrease with the increasing of adsorbent concentrations (X_1), bleaching temperature (X_2), bleaching period (X_3), interaction value between concentration and bleaching period (X_1X_3) and interaction value between bleaching temperature and period (X_2X_3). While the free fatty acid level tend to increase with the increasing of interaction value between adsorbent concentration and bleaching temperature (X_1X_2), quadratic interaction value of adsorbent concentration (X_1^2), quadratic interaction value of bleaching temperature (X_2^2) and quadratic interaction value of bleaching period (X_3^2). The following chart show us the effect of temperature and adsorbent concentration on FFA level. Both variables were chosen to determine its optimum point for operation condition.

Figure 2 shows circular contour lines with a red dot in the middle of the circle. Six central point on the contour is right at the center point of the circle. Blue area shows the lowest free fatty acids level. Blue area spreads to the lower right part of contour lines diagram. These condition indicates that the best response can not be obtained at the center point, so we must set all factors condition which can result response value in the blue area. The lowest free fatty acid level in the blue area was obtained at the optimum operation condition.

The main content of miracle filter powder (MFP) used in this study was magnesium silicate. Silicate compounds ability to lower FFA level is caused by silanol group (Si-O-H) on the surface of the silica adsorbent. Yang [9] stated that the silica surface chemistry is dominated by hydroxyl or silanol Si-O-H. Oxygen-carbonyl group at FFA reacts with hydrogen-silanol, so FFA molecules can be adsorbed to the adsorbent surface by forming hydrogen bonds with hydrogen-silanol group [10].

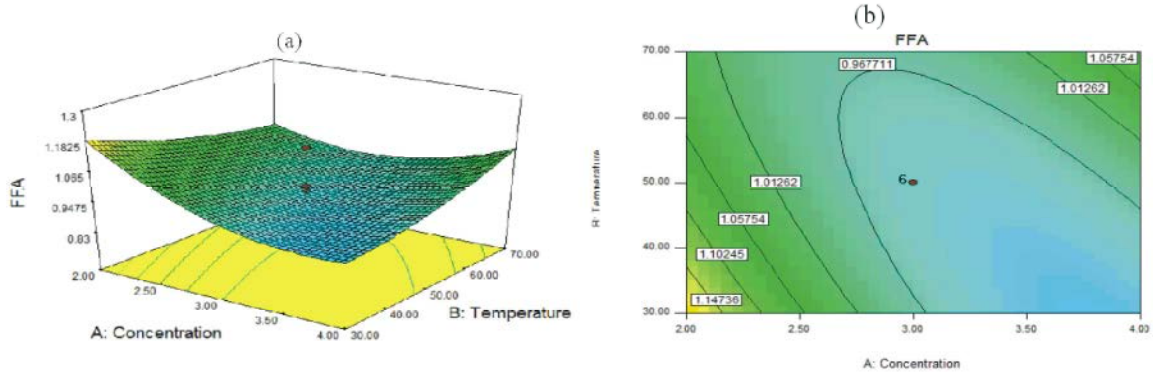

Fig. 2: Effect of adsorbent concentration and temperature of fish oil bleaching to FFA as response: chart (a) 3-Dimensional response and (b) contour

Table 7: Comparison of predictive response value for optimum condition to the actual value

			95% PI	
Response	Actual value	Predictive value	*Low*	*High*
Peroxide value	10.91± 0.05	10.19	8.13	12.24
Free Fatty Acid	1.05± 0.03	0.94	0.64	1.24

Validation of Optimum Condition: Recommended optimization solution by the program was a combination treatment of adsorbent concentration 3%, temperatures 50°C and 15 minutes of stirring (Bleaching period). Model will be considered as good and adequate if generated predictive response value approach the actual verification value [11].

After optimum point for operation condition was obtained, the next step was to validate the predictive response variable which was given. The program provides predictive value of the response, followed by a prediction interval of 95%. Prediction Interval (PI) is divided into two, namely 95% PI low and 95% PI high. PI low is the lowest value of the predicted interval. While the PI high is the highest value of the predicted interval. Definition of 95% in the PI is the trust value of individual observations by 95%. Prediction Interval values obtained from the program processed by Design Expert 7.0.0. Comparison of predictive response value for optimum condition to the actual value can be seen in Table 7.

Based on the validation test of the optimum optimum condition, the optimum operation condition resulted fish oil with PV at 10.91 meq / kg and FFA at 1.05%. When compared with the predicted response value provided by the program, the actual value did not differ significantly and was still in the 95% prediction interval. These condition indicates that the solution for fish oil bleaching optimization recommended by the program is good.

CONCLUSION

Optimum point for bleaching operation condition was a combination treatment of MFP 3%, adsorption temperature 50? and 15 minutes bleaching period. Based on the validation of optimum condition, its optimum condition could reduce 21.68% peroxide value and 30.68% FFA (free fatty acid) level.

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