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Application of Response Surface Methodology to Optimize the Extraction of Asian Catfish (*Clarias Batrachus*) Oil Using Superheated Steam Treatment

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Abstract Time and temperature parameters for yield of oil extracted from superheated steam treated catfish (Clarius batrachus) were optimized using response surface methodology. Response surface methodology (RSM) was applied to optimize the yield of catfish oil extraction by implementing Central Composite Design-Face centered. The optimal superheated steam treatment was obtained at 180°C and 18.57 minutes and considering operating convenience for the superheated steam oven, optimal values of variables were determined at 180°C for 18 minutes. Considering the operating convenience for superheated steam, the optimal values of variables were determined as follows: temperature: 180°C and time: 18 minutes. Under these conditions, the model predicted for the oil yield of *C. batrachus* was 5.27% with experimentally verified 5.27% ± 0.27. Lipid oxidation values under optimize fish oil extracted by superheated steam are within the acceptable standards for edible fish oils and much better compared to several extraction methods.

Keywords Superheated Steam, Oil Extraction, *Clarius Batrachus,* Temperature and Time

1. Introduction

Fish oil originated from marine fish was enriched with Omega-3, Omega-6, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and Polyunsaturated fatty acids (PUFA) most of which cannot be synthesized naturally by the human body thus, necessitating daily or weekly consumption [1]. However, with the decreasing number of marine fisheries resources, sourcing fish oil from marine resources has been discouraged and limited [2]. To overcome with this problem, freshwater fish has been seen as a potential source for fish oil. Compared to marine fish, freshwater fish contain high levels of C18 PUFA [3] meanwhile, ratio of ω-3:ω-6 fatty acids in total lipid of freshwater fish typically range from 0.5-3.8 [4]. The International Society for the study of Fatty Acids and Lipids (ISSFAL) recommends daily minimum intake of 0.22 g/day as Adequate Intakes (AIs) of DHA and EPA combination.

C. batrachus is highly cultured in South-East Asia in the Mekong and Chao Phraya basins, the Malaysian Peninsula, Sumatra, Java and Borneo. In 2012, total production from all species in freshwater culture system in Malaysia was 163,756.81 tonnes. The major freshwater species cultured were freshwater catfish with 46,522.98 tonnes contribute 28.41% from total freshwater production, followed by red tilapia 38,841.60 tonnes, river catfish 18,388.83 tonnes and black tilapia 12,713.40 tonnes [5].

Several studied on methods and conditions for fish oil extraction have been conducted in the past including solvent extraction method [6] supercritical fluid extraction [7], soxhlet method [8], wet and steam rendering methods [9], and wet pressing method [10]. Among these techniques, wet pressing method is widely used for oil extraction in the industrial scale as described by Food and Agriculture Organization [10]. This process involves cooking the raw material, pressing and lastly centrifugation or filtration of miscella to recover oil.

Superheated steam offers many advantages as drying agent, including reduce energy consumption, no fire or explosion risks, and improved quality of product i.e.: color, texture, and nutrients content. Bórquez et al. [11] stated that application of superheated steam on dried fish samples showed lower loses in n-3 fatty acid compared to conventional drying. Superheated steam has also been studied in drying of peanut (*Arachis hypogaea*) [12], fish meals [13], prawn [14], chicken sausage [15], and cocoa bean [16]. Heat treatment for oil extractions have been studied with microwave treatment on oil extraction from rapeseed [17], hazelnuts [18], and fish oil extraction [19]. Cold pressing oil extraction is a good alternative in comparison to the traditional high-temperature solvent extraction, albeit lower yields [20].

As far as it can be ascertained, there have been no published reports on the effects of superheated steam treatment on the extraction of fish oil. Therefore, this study aims to optimize the yield and evaluate the lipid oxidation of superheated steam treatment on Asian catfish (*Clarius batrachus*).

2. Materials and Methods

2.1. Material

Asian catfish (*Clarius batrachus*) samples were bought from TESCO, Gelugor, Pulau Pinang. The samples were immediately cut into small sizes, washed and stored in the freezer at -18°C before further processing.

2.2. Extraction Procedure

Samples were thawed overnight in a chiller at 7°C, before extraction process. Samples were lightly rinsed and then weighed in workable 600g batches, and those samples are heated in the super heated steam (Model AX-1500V (K), Sharp) according to statistical temperatures and time design. Hydraulic press (without heat) (Carver Laboratory press Model-M, Wabash, Indiana, USA) was used for the extraction process. Resulting miscella from hydraulic press was centrifuged to separate oil and finally the upper oil layer was collected using a pipette, stored in sample bottles and weighed. Samples were extracted once for each RSM experiment.

2.3. Experimental Design and Statistical Analysis

Pre-studied were conducted to get the range for the experiments. Software package, Design- Expert version 6.0.10 (Statease Inc., Minneapolis, USA) was applied in this optimization studied. To study the response pattern and establish a model, Central composite design Face centred (CCF) with two variables and three levels was selected. As shown in Table 1, the experiments were conducted according to Szydłowska et al. [21], consisting of 13 runs with two variables and five replicates of the central point for the estimation of pure error. Influence of factors on independent variables (Temperature: X_1 and Time: X_2) on the yield extraction (%) by superheated steam was described according to the equation below:

$$Y= b_0 + b_1X_1 + b_{12}X_1X_2 + b_{11} (X_1)^2 + b_{22} (X_2)^2 \quad (1)$$

Where y is the dependent variable predicted by the model (response); b_0 is the constant coefficient of intercept; b_1 and b_2 are the linear effects, b_{11} and b_{22} are the quadratic effects, b_{12} is the linear-by-linear interaction. Temperature (°C) and time (%) are represented by X_1 and X_2, respectively, as the coded independent variables.

Table 1. Independent variables, coded level and experimental matrix for the optimization of superheated steam treatment for oil extraction.

Independent variables	**Coded level Xi**		
	-1	0	+1
X_1: Temperature (°C)	120	150	180
X_2 : Time (minutes)	5	15	25

Run	Coded level	
	X_1	X_2
1	0	0
2	-1	-1
3	0	0
4	0	0
5	+1	-1
6	-1	+1
7	+1	+1
8	0	+1
9	-1	0
10	0	0
11	0	0
12	+1	0
13	0	-1

2.4. Peroxide Value

Peroxide value was determined according to method AOAC method 965.33 (AOAC, 2000). 5.00g of catfish oil was dissolved in 30mL of CH_3COOH-$CHCL_3$. 0.5mL of saturated KI solution was added and shakes for 1 minute before mixed it together with 30mL of H_20. The mixture was slowly titrated with 0.1M $Na_2S_2O_3$ until yellow colour almost disappeared. Finally, 0.5mL of 1% starch solution was added and run the titration process until blue colour totally gone. Samples was determined in triplicate and expressed as [miliequivalent peroxide/ kg oil or fat (mEq/kg)].

PV = S × M × 1000/ g Sample

Where:

S = Volume of $Na_2S_2O_3$ (mL)

M = Molarity $Na_2S_2O_3$ solution (M)

2.5. P-anisidine Value

P-anisidine value was determined according to AOCS Cd-18-90 method. 1.00g of oil samples was mixed with 25mL of isooctane to produce mixture A. Absorbance for mixture A was read at 350nm by using spectrophotometer (UVmini-1240, UV-VIS Spectrophotometer, Shimadzu, Columbia, USA), with isooctane as a blank. 5mL of mixture A was added with 1mL of 0.25% p-anisidine and stored in the dark for 10 minutes to produce mixture B. Lastly, absorbance for mixture B was read at 350nm. P-anisidine analysis was done in triplicate and calculated as follow:

P-AV = 25 × (1.2 (AS) – AB) / W

Where:

AS = Absorbance of the solution after react with P-anisidine

AB = Absorbance of the solution before react with P-anisidine

W = Weight of sample (g)

2.6. Fatty acids (Free) and Acid value

Analysis for FFA and Acids value was determined according to AOAC method 940.28. 7.05g of catfish oil was added with 50mL of 95% ethanol. Mixture was then titrated with 0.25N of NaOH solution with vigorous shaking until permanent faint pink appeared and persist for 1 minute. Percentage for FFA was expressed as oleic acid. The formulation as shown below:

% FFA = (V × N × 28.2) / W

Where:

V = Volume of 0.25N NaOH (mL)

N = Normality of NaOH (N)

W = Weight sample (g)

Acid value = % FFA × 1.99

2.7. Iodine Value

AOAC method 993.20 was used to determine the iodine value. 0.21-0.26g of oil sample was mixed with 15mL of mixture cyclohexane and acetic acid (1:1). 25mL of Wijs solution was added and immediately stored in the dark at 25°C for 1 hour. After 1 hour storage, 20mL of saturated KI and 150mL of distilled water was added. Then, mixture was titrated with 0.1N $Na_2S_2O_3$ until yellow colour disappeared. 2mL starch was added and continues titration until blue colour was gone. The titration was performed with blank (without sample). Iodine value was calculated as follows:

IV = [(B-S) × N × 12.69] / W

B = Titration of blank (mL)

S = Titration of sample (mL)

N = Normality of Na2S2O3

W = Weight sample (g)

3. Results and Discussions

Effect of temperature (X_1) and time (X_2) of superheated steam on the experimental value and predictive value for yield of oil extraction (reponse) was shown in Table 2. Table 3 describe the statistic analysis for the linear, quadratic and the interactions between those two variables (X_1 and X_2) on the response values (Y) meanwhile, figure 1 and figure 2 illustrate response surface quadratic model and fitted line plot between prediction and actual values respectively.

3.1. Model Fitting

Myers and Montgomery [22], suggested that the model adequacies were checked by their R^2, adjusted R^2, predicted R^2, coefficient of variation (CV), and prediction error sum of squares (PRESS). Significance model terms might exhibits a good fit, which makes the checking of the particular model adequacy essential [23, 24, 25].

Lack-of-fit is measuring how well the models fit the data. Base on the result in Table 3, it showed lack of fit have not significant with p-value 0.8242, meaning that this model was sufficiently accurate predicting the relevant response. Results on this studied shows R^2 value for this response variable is 0.969, in which revealing that the model could not explain only 3% of the overall effects, thus ensuring a close agreement of the fitting the regression models to the experimental data.R^2 is not considered as the main point to imply the accuracy of the model, because in addition of the variable to the model will increase the value of R^2. Due to this matter, adj-R^2 is more appropriate to evaluate the model if its value is more than 90% [26]. Result for Adj-R^2 and Pre R^2 shows value 0.9481 and 0.9252 respectively with range value 0.02. All those data obtained will strengthen the accuracy of the model.

Table 2. Experimental value and predictive value for yield of oil extraction from Asian catfish (*Clarius batrachus*)

Run	Coded Level		Oil Yield (%)	
	X_1	X_2	*Experimental Value*	*Predictive Value*
1	0	0	5.17	5.27
2	-1	-1	2.04	1.97
3	0	0	5.88	5.27
4	0	0	4.98	5.27
5	+1	-1	3.27	3.23
6	-1	+1	6.14	6.05
7	+1	+1	4.84	4.77
8	0	+1	5.44	5.60
9	-1	0	4.94	5.10
10	0	0	5.33	5.27
11	0	0	5.27	5.27
12	+1	0	4.98	5.09
13	0	-1	2.68	2.79

Table 3. ANOVA for Response Surface Quadratic Model on oil extraction by superheated steam treatment.

Source	DF	Sum of Squares	Mean Squares	F value	p-value Prob > F
Model	5	17.83	3.57	44.85	< 0.0001 significant
Residual	7	0.56	0.080		
Cor Total	12	18.39			
Lack-of-fit	3	0.10	0.034	0.30	0.8242 not significant
Pure error	4	0.45	0.11		
R^2	0.9697				
Adj R^2	0.9481				
Pred R^2	0.9252				
C.V. %	6.01				
Adeq Precisior	21.270				

3.2. Analysis of Response Surfaces

According to the equation, effects of two independent variables, A: temperature and B: time on the yield obtained for extraction of fish oil by superheated steam treatment was illustrated using surface response and contour plots of the Quadric polynomial models (Figure 1). As can be seen in Figure 1, response was proportionally increased with the increasing temperature and time. But at some point, the response's value starts to decrease with the rising of the experimental value. It was observed that, lowest oil production was found at lowest steam temperature with shortest treatment time. Result showed that at optimum Superheated steam conditions (temperature: 180°C, time: 15 minutes), oil obtained from the extraction of *C.batrachus* was 5.27% ± 0.27. Ramakrishnan et al., [27] stated that initial heating on the raw samples played a key point to optimise the fish oil yield, and it would be more effective without addition of buffer during the process. Heat or enzymatic treatments during extraction process will denaturized the protein matrix (intracellular oil) [28], which helps in maximize the oil yield extraction.

Several studies have been done on oil extraction treated by superheated steam including extraction of the essential oil of thyme and black pepper [29], and rice oil from rice bran [30]. However, there is a lack of studies on production of oil extraction from fresh water fish by superheated steam treatments. Effect of optimized conditions by supercritical fluid extraction on the oil extraction from viscera African catfish showed oil extraction yield was 67.0% (dry weight basis) [31], solvent extraction process on C.batrachus resulted in 6.91% oil recovered [32], meanwhile crude oil obtained by enzymatic hydrolysis extraction of African catfish viscera was 14.3% ± 1.4 [33]. Ferdosh et al., [8] reported oil yield obtain by Soxhlet extraction from Thunnus tonggol, Euthynnus affinis, and Auxis thazard viscera was at 13.5 ± 0.6, 16.1 ± 0.8 and 16.8 ± 1.2 respectively.

Although previous results were higher compared to oil yield gained from this study, there were some drawback effects in these methods i.e economic reasons for supercritical fluid extraction [34], toxic solvent residues in the final product that cause adverse effects on human health , protein denaturation and loss of functional properties for organic solvent extraction [35, 36, 37]. On the other hand, Soxhlet extraction method is time consuming and requires a relatively dry sample to enable solvent to penetrate into the samples [38].

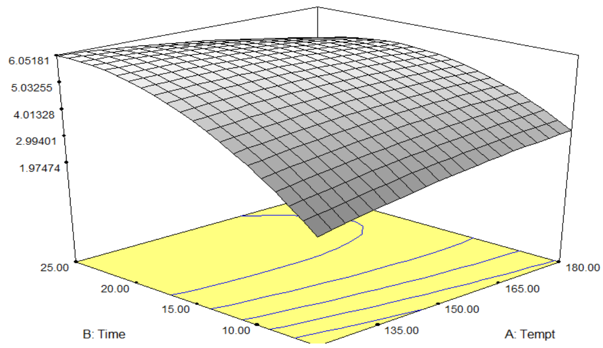

Figure 1. Response surface plots and contour plots for the interactive effects of inlet temperature and pump on the yield of oil extraction by superheated steam treatment.

The accuracy of the response surface equations can be proved by comparing experimental value and predicted data. The comparison was performed by generating a fitted-line plot for the results obtained, showing how close it was or how far it deviated from the fitted line. As shown in Figure 2, agreement between predicted values and experimental values proved that the response surface models in this research were adequate for predicting the yield of oil extraction by superheated steam treatment.

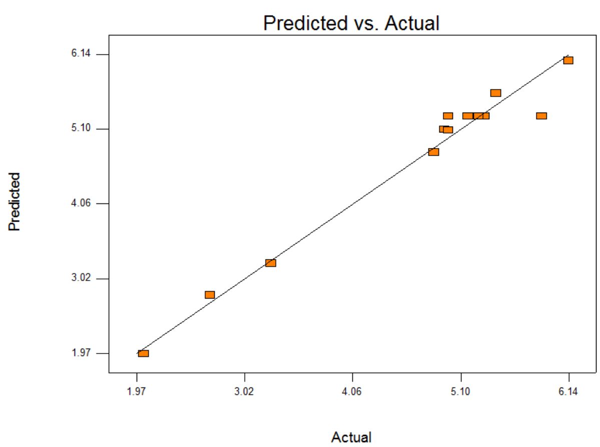

Figure 2. Fitted line plot indicating the closeness between predicted values and experimental values for yield of oil extraction from African catfish by superheated steam treatment.

3.3. Validation of Predictive Model

The optimum values were found by solving the regression equation analytically. The optimal superheated steam treatments were calculated as follows: temperature at 180°C and time at 18.57 minutes. Considering the operating convenience for super heated steam (Model AX-1500V (K), Sharp), the optimal values of variables were determined as follows; temperature at 180°C and time at 18 minutes. To validate the predicted model, tests were carried out at optimal conditions for temperature and time. The predicted response (oil yield) is 5.27% was experimentally verified at 5.27% ± 0.27.

3.4. Lipid Oxidation

Numerous different standards with varies acceptable levels for fish oil quality have been established. The objectives for all those standards are to control the quality of fish oil properties from potential biological and chemical hazards related to oxidation. High level of PUFA causes fish oil easily to hydrolytic spoilage especially oxidative deterioration [39]. Oxidation of lipids lead to some disadvantage including rancid odours and flavours, reducing nutritional quality and safety [40], to be worst it may cause on health hazards [41].

Peroxide Value (PV) is primary oxidation products that give sign for initial rancidity in the oil. According to European Food Saferty Authority (EFSA) [1], peroxide value, PV, and acids value, AV, are recommended as chemical methods for determination of the oxidative status for crude fish oil and refined fish oil, as these methods are commonly used in the industry. U.S Food and Drugs Administration Generally Recognised as Safe (FDA GRAS)[42,43], suggested that PV should be <2.5 mEq/kg and AV <1.0 %, meanwhile by Global Organization for EPA and DHA Omega-3s (GOED) [44] stated PV should <5 meq/kg whereas in food products should not more than 2 meq/kg. Refer to the table 4; PV and AV show values 1.98 ± 0.55 mEq/kg and 1.75% ± 0.15 respectively which meet the level of standard by FDA GRAS and GOED . Studied by A.K Rai et al., [45] on fermentation ensilaging of fresh water fish viscera have range between 30.5–121.0 mg KOH/g oil for AV.

Table 4. Analysis of lipid oxidation of fish oil extraction by using superheated steam treatment.

Analysis	Results
Peroxide Value	1.98 ± 0.55 meq/kg
P-anisidine Value	3.49 ± 0.15 mmol/kg
Free Fatty Acids (% Oleic acid)	0.88% ± 0.08
Acid Value	1.75% ± 0.15
Iodine Value	75.42% ± 0.51

P-anisidine value is secondary products from the oxidation process that determines the decreasing of peroxide values but increasing of anisidine value. It is represented by further degradation of lipids through a radical oxidation process initiated by hydroperoxides. This studies shows P-anisidine value was 3.49 ± 0.15 mmol/kg in the range to standard by FDA GRAS <20 [42, 43, 46] and 4-60 [47]. Noriega-Rodrı´guez et al., [48] stated decreasing p-anisidine value (from 3.67 to 1.63 mmol/kg) had been observed on refining process sardine oil (S. sagax). P-anisidine value gained by ensilage crude oil from carp (*Cyprinus carpio*) viscera was10.3 ± 0.3 [49] a bit higher compared to superheated steam treatment.

Free Fatty Acids are a key of hydrolytic degradation associated with off flavor and textural changes. Acceptable levels of FFA content in fish oil ranges between 1.8%-3.5% [33], 2–5% [47] with maximum acceptability at 4% [50].Value of free fatty acids for fish oil obtained from *C.batrachus* treated by superheated steam was 0.88% ± 0.08 much lower compared to acceptable levels. In contrast, results on this studies was low compared to FFA oil extracted by different solvent extraction of Atlantic salmon skin (0.60–1.19%) [50], catfish oil; heat treatment (2.8 ± 0.04), and enzymatic hydrolysis (3.55 ± 0.09)[33].

Iodine value is a method to determine the degree of unsaturation of tryglycerides. Base on this study, Iodine value for oil extraction of catfish by using superheated steam treatment was 75.42% ± 0.51 below than typical iodine values for fish oil, 120-180 [51], iodine value by Bimbo [47], and range value from fresh water fish viscera 95–118 [45].

4. Conclusion

Base on the experiment that had been done, the experimental values agreed with the predicted values, using analysis of variance, indicating an excellent fit of the model used and the success of response surface methodology for modelling extraction of superheated steam treatment on the oil extraction from the Asian catfish (*Clarius batrachus*). The optimal values of variables were determined at temperature 180°C with treatment time at 18 minutes was predicted response as 5.27% and experimentally verified 5.27%±0.27. Fish oil extracted by superheated steam had lower lipid oxidation; peroxide value, p-anisidine value, free fatty acids, acids value and iodine value compared to several standards with value 1.98 ± 0.55 meq/kg, 3.49 ± 0.15, 0.88% ± 0.08, 1.75% ± 0.15 and 75.42% ± 0.51 respectively. These values are within the acceptable standards for edible fish oils and much better compared to several extraction methods. It suggested that superheated steam treatment can be used to help in oil extraction from the *C. batrachus*.

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