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EXTRACTION OPTIMIZATION OF EEL (*Anguilla bicolor*) OIL USING REFLUX AND ULTRASOUND-ASSISTED EXTRACTION METHODS

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Abstract

Eel (*Anguilla bicolor*) is a freshwater fish that contains polyunsaturated fatty acids including omega-3 fatty acids and making it a potential source for food enrichment. This study aimed to determine the optimum conditions for eel oil extraction using reflux and ultrasonic-assisted extraction methods and to compare the efficiency of both techniques. A central composite design within response surface methodology (RSM) was employed to optimize the extraction parameters. Reaction time (30, 60, and 90 minutes) and solvent-to-solid ratio (5:1, 10:1, and 15:1) were selected as independent variables, while oil yield was used as the response variable. RSM analysis revealed that both reaction time and solvent ratio significantly influenced oil yield ($p<0.05$). The UAE method produced a higher optimum yield (47.53%) than the reflux method (45.87%). The optimum extraction conditions for UAE were achieved at a solvent-to-solid ratio of 11.21:1 and 69.50 minutes, while the reflux extraction was optimized at 12.21:1 and 70.55 minutes. Replication of extractions confirmed consistent yields of 47.7% for UAE and 45.6% for reflux. Fatty acid analysis indicated that oil from reflux extraction contained 31.15% saturated and 68.38% unsaturated fatty acids, while UAE yielded 32.75% saturated and 66.76% unsaturated fatty acids. Although UAE was more efficient in terms of yield, time, and solvent use, the reflux method resulted eel oil with higher concentrations of polyunsaturated fatty acids, including α-linolenic acid, eicosatrienoic acid, and eicosapentaenoic acid. This study proves that optimizing the extraction of both methods using RSM has produced eel oil with high yield and PUFAs content.

Keywords: health benefit, oil extraction, omega-3 fatty acids, polyunsaturated fatty acids, response surface methodology

Optimasi Ekstraksi Minyak Ikan Sidat (*Anguilla bicolor*) menggunakan Metode Refluks dan Ekstraksi Berbantuan Gelombang Ultrasonik

Abstrak

Ikan sidat (*Anguilla bicolor*) merupakan ikan air tawar yang mengandung asam lemak omega-3 dan lemak tak jenuh ganda, sehingga berpotensi sebagai sumber pengayaan pangan fungsional. Penelitian ini bertujuan untuk menentukan kondisi optimum ekstraksi minyak ikan sidat menggunakan metode refluks dan ekstraksi berbantuan gelombang ultrasonik (UAE) serta membandingkan efisiensi kedua metode tersebut. Desain komposit pusat dalam rancangan metode respon permukaan digunakan untuk mengoptimalkan parameter ekstraksi. Waktu reaksi (30, 60, dan 90 menit) serta rasio pelarut terhadap bahan (5:1, 10:1, dan

15:1) ditetapkan sebagai variabel bebas, sedangkan rendemen minyak digunakan sebagai variabel respons. Analisis RSM berbasis ANOVA menunjukkan bahwa waktu reaksi dan rasio pelarut secara signifikan memengaruhi rendemen minyak ($p<0{,}05$). Metode UAE menghasilkan rendemen optimum yang lebih tinggi (47,53%) dibandingkan metode refluks (45,87%). Kondisi optimum UAE diperoleh pada rasio pelarut terhadap bahan sebesar 11,21:1 dan waktu ekstraksi 69,50 menit, sedangkan ekstraksi refluks optimum pada rasio 12,21:1 dan waktu 70,55 menit. Ekstraksi ulangan menunjukkan rendemen yang konsisten, yaitu 47,7% untuk UAE dan 45,6% untuk refluks. Analisis asam lemak menunjukkan bahwa minyak hasil ekstraksi refluks mengandung 31,15% asam lemak jenuh dan 68,38% asam lemak tak jenuh, sedangkan metode UAE menghasilkan 32,75% asam lemak jenuh dan 66,76% asam lemak tak jenuh. Meskipun metode UAE lebih efisien dari segi rendemen, waktu, dan penggunaan pelarut, metode refluks menghasilkan minyak ikan sidat dengan konsentrasi asam lemak tak jenuh ganda yang bermanfaat lebih tinggi, termasuk asam α-linolenat, asam eikosatrienoat, dan asam eikosapentaenoat. Temuan ini menunjukkan bahwa pemilihan metode ekstraksi perlu disesuaikan dengan tujuan aplikasi fungsional minyak ikan sidat.

Kata kunci: asam lemak omega-3, asam lemak tidak jenuh, ekstraksi minyak, manfaat kesehatan, metode respons permukaan

INTRODUCTION

Indonesia has a wide variety of nutritious and beneficial freshwater fish species. According to recent data from the Ministry of Marine Affairs and Fisheries (KKP), national fish consumption in Indonesia reached approximately 58.9 kg per capita per year in 2024 (Kementerian Kelautan dan Perikanan, 2025). This highlights the important role of fish, including freshwater species, in the Indonesian diet. Among freshwater fish, eels (*Anguilla bicolor*) are known for their distinctive sensory characteristics and favorable nutritional composition (Muhtiani *et al.*, 2020).

Fish oil, derived from fish and/or fish by-products, is abundant in unsaturated fatty acids, particularly omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Aitta *et al.*, 2021). Numerous studies have demonstrated the advantages of omega-3 fatty acids in the prevention and treatment of illnesses. Recent studies have revealed that the DHA-to-EPA ratio in fish oil is crucial for the health of various age groups (Alfio *et al.*, 2021).

Researchers have found potential benefits of eel and its oil for human health. Indonesian shortfin eel contains diverse fatty acids, including omega-3 fatty acids, supporting its potential as a functional food source (Rawendra *et al.*, 2021). Eels may originate from both wild and farm sources. A study on farmed eels in West Java reported that eels contain 16.78% protein, 1.09% ash, 13.26% fat, 65.51% moisture, 25.10% saturated fatty acids (SFA), 30.10% monounsaturated fatty acids (MUFA), and 17.87% polyunsaturated fatty acids (PUFAs) (9.74% DHA and 1.79% EPA) (Nafsiyah *et al.*, 2018). This supports its potential as a valuable food source.

There are two main methods of eel oil extraction: reflux extraction and ultrasonic-assisted extraction (UAE). The latter has been found to be more efficient, resulting in higher yields in shorter times. Reflux extraction, the most conventional method for extracting eel oil (Sahriawati & Daud, 2016), utilizes high temperatures and specific solvents, commonly ethanol or n-hexane, to thoroughly capture all bioactive compounds (Yasin *et al.*, 2021), including omega-3 fatty acids, mono- and polyunsaturated fatty acids, steroids, squalene, and phospholipids (Febrianta & Rawendra, 2019). This method is quite effective and can produce higher yields of *Anguilla bicolor* oil (1.49%) compared to *Monopterus albus* oil (0.056%) (Sandita *et al.*, 2015). Oil extraction using ultrasonic waves is also more effective and efficient for isolating solid components in animal and plant tissues (Bendicho & Lavilla, 2018).

In addition to resulting in a high oil yield, the UAE method increases oil quantity and quality compared to the reflux extraction method (Gulzar & Benjakul, 2018). Previous studies comparing the two methods for the extraction of rapeseed oil found that the UAE approach took only five minutes to achieve an extraction rate of 19.2±0.04, which then steadily decreased, while the extraction rates from the reflux and impregnation techniques were 21.32±0.05 and 20.44±0.05, respectively (Shen *et al*., 2023). The fish oil extraction process is influenced by three main factors: the type of solvent used, reaction time, and the ratio of solvent to solids (Sahriawati & Daud, 2016). Response surface methodology (RSM) can be used to understand how these factors interact and impact the overall extraction process.

Previous studies on eel oil have primarily focused on nutritional characterization and conventional extraction approaches. A systematic optimization of the extraction process and direct comparison between reflux and ultrasound-assisted extraction methods using response surface methodology remains limited. Therefore, this study addresses this research gap by optimizing and comparing reflux and UAE techniques to obtain eel oil with a high yield and fatty acid quality.

RSM with a central composite design (CCD) was applied as an efficient approach to optimize eel oil extraction parameters using a limited number of experimental runs. Optimization was conducted by comparing the reflux and UAE methods to achieve maximum oil yield. The quality of eel oil was further evaluated based on its chemical characteristics and its fatty acid composition. This study aimed to determine the optimum conditions for eel oil extraction using reflux and ultrasonic-assisted extraction methods and to compare the efficiency of both techniques.

MATERIALS AND METHODS

Sample Preparation

Sample preparation was performed following previously reported methods with modifications (Laksmiani *et al*., 2015; Sahriawati & Daud, 2016; Mendez & Concha, 2018). Fresh eel was cleaned by removing the head, skin, gills, stomach, and bones to obtain fish fillets. The fillet was cut into small dices (2×2 cm), washed several times, drained, and steamed for 5 min. The steamed fish fillets were dried at 40˚C for 16 h and then pulverized using a blender. Each sample was measured ±10 g, filled into a plastic clip, and stored in a desiccator before analysis.

The primary material used in this study was eel from a cultivation pond in Bogor Regency, West Java. The eels had an average body weight of 250 g and an average total length of 52 cm. All chemicals used were of analytical grade. Distilled water was used to wash the eel fillets before drying.

Extraction Procedure for Cultured Eel Oil

Oil extraction was performed using the reflux and UAE methods. The extraction procedure was developed based on the commonly reported reflux and ultrasound-assisted extraction principles in fish oil studies. Therefore, the zmethod described as adapted and modified from previously reported methods of UAE (Mendez & Concha, 2018) and reflux extraction (Sabar *et al*., 2015; Sandita *et al*., 2015; Sahriawati & Daud, 2016). The schematic design of the experimental study is shown in Figure 1.

The reflux extraction process began by placing 10 g of dried eel fillet into a three-neck flask. Subsequently, n-hexane was poured into the flask using three solvent-to-solid ratios of 5:1, 10:1, and 15:1 mL/g. The extraction process was conducted at a temperature of ±70˚C with different reaction times of 30, 60, and 90 min. The UAE extraction process followed the same procedure. The samples were subjected to ultrasonication (Digital Ultrasonic Cleaner, CDS-300, 42 KHz, China). During ultrasonication, the probe was submerged in the center of the solution approximately 2 cm below the liquid surface, and the solution was cooled in a thermostatic water bath to maintain a constant temperature of 70°C. The temperature of ±70˚C was monitored during the extraction process. The flask was removed from the ultrasonic bath and allowed to stand for 5 min at room temperature. The results of the two extraction processes were filtered using Whatman 41 filter paper. The solvent was then evaporated using a rotary evaporator (EYELA–OSB 2000, Japan). The evaporation process was carried out at 60˚C for 7 min, and the resulting eel oil was placed in a 10 mL dark glass bottle.

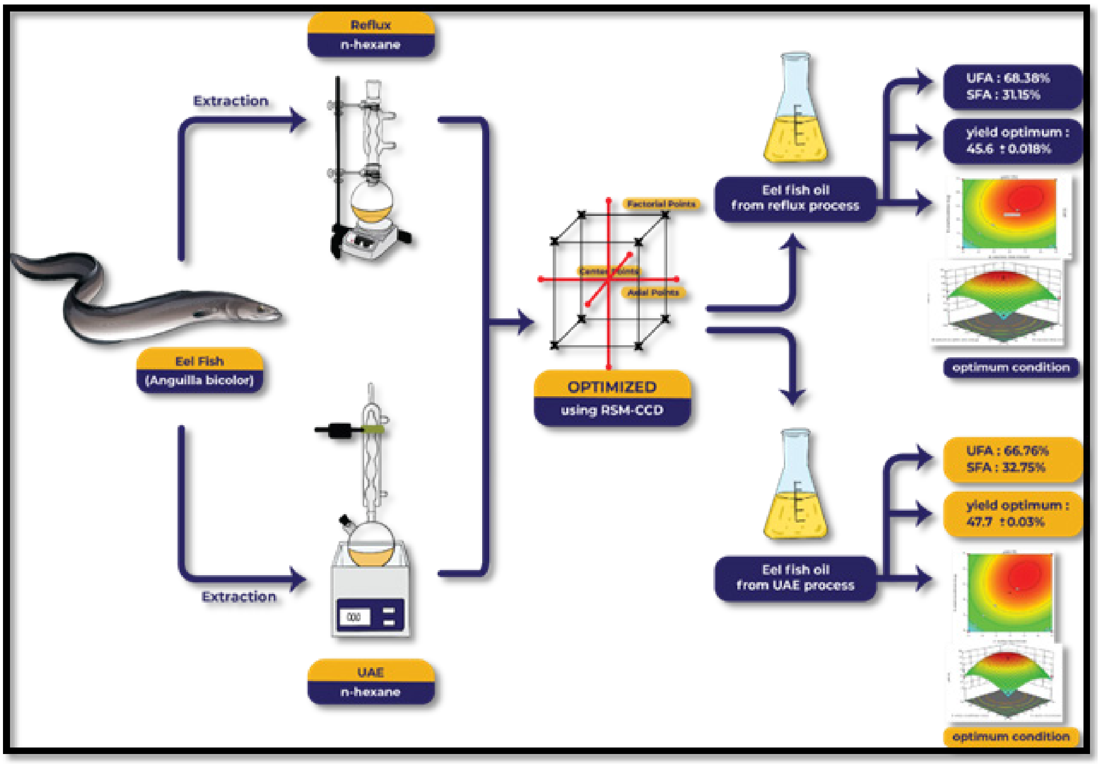

Figure 1 The schematic design of experimental study

Optimization Using RSM Centered Composite Design

Eel oil extraction was optimized using response surface methodology (RSM) with a central composite design (CCD) to model and optimize process variables (Bayuo *et al*., 2020; Dharmegowda *et al.*, 2023; Limmun *et al*., 2024). Three reaction times (30, 60, and 90 min) were selected as the first independent variable (X_1), and the ratio of solvent-to-solids (5:1, 10:1, and 15:1 mL/g) was chosen as the second independent variable (X_2). The determination of independent variables, treatment codes, and experimental design is presented in Table 1. Each independent variable was determined by the lower and upper limits tolerated to determine the optimum conditions for the eel oil extraction process. The values are symbolized as (-) α and (+) α (Table 1) to determine the number of experimental designs conducted.

RSM analysis was conducted using Design Expert software version 12. Reaction time (X_1) and solvent-to-solid ratio (X_2) were selected as independent variables, and oil yield was used as the response variable (Y). Analysis of variance (ANOVA) was performed as an integral part of the RSM to evaluate the

Table 1 Determination of independent variables and treatment codes in extraction process

Independent variables	Symbol	Range and level				
		-α	-1	0	+1	+α
Reflux process						
Reaction time (min)	X_1	18	30	60	90	102
Solvent to solid ratio (mL/g)	X_2	3:1	5:1	10:1	15:1	17:1
Ultrasound assisted extraction process						
Reaction time (min)	X_1	18	30	60	90	102
Solvent to solid ratio (mL/g)	X_2	3:1	5:1	10:1	15:1	17:1

significance of the regression model, individual factors, and their interactions. The optimum surface response was calculated based on the stationary points, contour plots, and surface plots. A second-order polynomial equation was used to describe the relationship between the independent and response variables (Eq. 1) (Mašković *et al.*, 2024).

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_{21}+\beta_{22}X_{22} +\beta_{12}X_1X_2+e \qquad (1)$$

where
β_0 = a constant parameter
β_1 β_2 = a linear coefficients
β_{11} β_{12} = a quadratic coefficients
β_{12} = the interaction coefficients
e = the random error

The optimum extraction conditions predicted by the RSM model were validated using three independent experimental replicates.

Chemical and Fatty Acid Analysis

Eel oil obtained under optimum extraction conditions was analyzed for the following quality parameters: moisture, acid, peroxide, iodine, saponification, and free fatty acid (FFA) values. Chemical analyses, including acid value (AOAC, 2005), peroxide value (AOAC, 2005), iodine value (AOAC, 2016), moisture content (AOAC, 2005), and fatty acid methyl ester (FAME) analysis (AOAC, 2005). The free fatty acid (AOCS, 2003) and saponification values (AOCS, 2003) were determined according to the AOCS methods. Ca 5a-40 and Cd 3-25, respectively.

Fatty acid composition was determined using gas chromatography (Shimadzu GC-2030) equipped with a flame ionization detector (FID) and a capillary column (Agilent Dura Bond (DB) 23.60 m×0.25 mm×0.25 µm). Esterification was performed according to the AOAC (2005). Briefly, the transformation of carboxylic acids into esters was initiated by weighing 0.1 g of the sample into a 50 mL flask, followed by the addition of 4 mL of methanolic sodium hydroxide (NaOH). The mixture was heated in a water bath for 5–10 min until completely dissolved. Gradually, 5 mL of BF_3 was added, and 2 mL of heptane was introduced while heating was continued for 1 min. After cooling, 20 mL of saturated sodium chloride (NaCl) was added, and the mixture was vortexed. Then, 1 mL of the heptane solution (upper layer) was transferred to a sealed test tube. The test solution was formulated for injection into the GC under designated conditions. The injector temperature was at 240°C and detector temperatures were both set at 240°C, with helium as the carrier gas. The column temperature was maintained at 185°C, with a hold duration of 15 min and a split ratio of 1:100.

RESULT AND DISCUSSION

Optimization of Extraction Process

Reflux and UAE extraction procedures involve physicochemical mechanisms for extracting bioactive compounds from natural materials. Reflux extraction employs elevated temperatures and solvents to increase solubility, facilitating the extraction of isolated active ingredients. Ultrasonic extraction utilizes the acoustic cavitation effect, eliminating the need for elevated extraction temperatures (Oprescu *et al.*, 2022). These two methods undoubtedly influence the yield outcomes, both quality and quantity. The yields of the eel oil obtained are presented in Tables 2 and 3.

The number of trials (runs) of the two extraction optimizations was adjusted to collect the necessary data to optimize each process. In the reflux extraction, 13 trials were conducted to achieve optimum conditions, whereas in the UAE extraction process, 16 trials were conducted. This was based on computational results that showed a discrepancy between the suggested and proposed models, where the number of center points was increased by three; thus, the experiment was performed 16 times. The addition of center points in the UAE aimed to improve the accuracy of the optimization model by providing more information about the relationship between the independent and response variables (Tajadodi *et al.*, 2024). Furthermore, variable interactions can be used as references to calculate the uncertainty in the experiment (Nguyen *et al.*, 2024) (Table 2).

Table 2 Interaction between independent and response variables

Run	Reaction time (min)	Solvent to solid ratio (mL/g)	Reflux yield (%)	UAE yield (%)
1	60	10:1	44.3	45.3
2	60	3:1	20.6	23.5
3	90	5:1	30.5	31.1
4	60	17:1	40.8	43.8
5	102	10:1	36.1	37.3
6	90	15:1	40.3	43.5
7	30	5:1	28.7	29.8
8	18	10:1	26.1	27.8
9	60	10:1	41.9	41.8
10	60	10:1	42.4	47.5
11	60	10:1	46.1	45.8
12	30	15:1	29.3	30.1
13	60	10:1	45.3	47.2
14	60	10:1	-	46.9
15	60	10:1	-	45.8
16	60	10:1	-	47.3

The optimum extraction conditions for eel oil using the reflux and UAE methods were determined in several stages: oil yield response determination, response model analysis, analysis of variance (ANOVA) and the influence of variables on the response, oil yield optimization in central composite design, and verification of predicted values with experimental values. Analysis using the RSM approach produced data in the form of predictions regarding the effect of independent variables on the resulting response (Behera *et al.*, 2018). As shown in Table 2, the highest oil yield of reflux extraction optimization was 46.1%, while that of UAE was 47.5% with a solvent-to-solid ratio of 10:1 and extraction process of 60 min. The lowest oil yield for UAE extraction was 23.5%, while that for reflux extraction was 20.6% with a 3:1 mL/g ratio and 60 min extraction time. Both extraction processes were optimized using multiple regression analysis. The extraction yield of eel oil by the reflux method was calculated using Eq. 2 (Bayuo *et al.*, 2020), while the UAE extraction process was performed using Eq. 3 (Behera *et al.*, 2018).

$$Y = -13.67731 + 0.788926X_1 + 0.519418X_2 + 0.001533X_1X_2 - 0.006917X_{12} - 0.002570X_2 \quad (2)$$

$$Y = -9.68586 + 0.791397X_1 + 0.464020X_2 + 0.002017X_1X_2 - 0.007299X_{12} - 0.002407X_2 \quad (3)$$

Applying the quadratic model to the reflux and UAE processes significantly affects the oil yield ($p<0.05$), indicating that the model is effective in predicting the response value. The F value for the reflux process was 20.36 and 34.46 for the UAE process. This indicates the variables of both extraction processes including reaction time ($p<0.05$) and solvent-to-solids ratio ($p<0.05$) were statistically significant, with the UAE model demonstrating a stronger ability to explain the variation in oil yield. Model deviation was assessed using the lack of fit (LOF) value, which was 0.0562 ($p>0.05$), meaning that the experimental data showed no significant lack of fit which can be seen in Table 3.

The higher the F value, the more appropriate the use of the quadratic model

Table 3 Anova results for quadratic model of the reflux optimization process and UAE

Source	Sum of square		Df		Mean square		F-value		*p*-value	
	Reflux	UAE	Reflux	UAE	Reflux	UAE	Reflux	UAE	Reflux	UAE
Model	794.24	984.87	5	5	158.85	196.97	20.36	34.46	0.0005	<0.0001*
X_1-reaction time	90.73	98.95	1	1	90.73	98.95	11.63	17.31	0.0113	0.0019
X_2-ratio	189.80	214.34	1	1	189.80	214.34	24.32	37.49	0.0017	0.0001
$X_1 X_2$	21.16	36.60	1	1	21.16	36.60	2.71	6.40	0.1436	0.0299
X_{12}	269.57	345.19	1	1	269.57	345.19	34.55	60.38	0.0006	<0.0001
X_{22}	287.17	289.80	1	1	287.17	289.80	36.80	50.70	0.0005	<0.0001
Residuals	54.62	57.17	7	10	7.80	5.72				
Lack of fit	44.86	32.79	3	3	14.95	10.93	6.13	3.14	0.0562	0.0963**
Pure error	9.76	24.38	4	7	2.44	3.48				
Cast Total	848.86	1042.04	12	15						

* = significant, ** = not significant

suggested by RSM (Limmun *et al.*, 2024). The greater the lack of fit value (*p*>0.05), the better, meaning it is not significantly different from the specified significance level (α=5 percent) (Bayuo *et al.*, 2020). Overall, these results suggest that reaction time and the use of solvent-to-solids ratio have a statistically significant influence on the amount of yield produced in the extraction process (Sulaiman *et al.*, 2017).

The quality of the fit of the optimization model was determined using the coefficient of determination (R2). The R2 value obtained for the reflux process was 0.9357 and in the UAE process it was 0.9451 (Table 4). These values suggest that the quadratic model effective-ly captures the relationship between the adjusted parameters of both extraction processes (Dharmegowda *et al.*, 2023). Additionally, the adjusted coefficient of determination (adjusted R2) was used to support the significance of the model selection (Varsha *et al.*, 2024).

The maximum oil yields obtained in the present study were 46.1% for reflux extraction and 47.5% for ultrasound-assisted extraction (UAE), using a solvent-to-solid ratio of 10:1 and an extraction time of 60 minutes. These yields are significantly higher than those reported in previous studies; for instance, extraction using diethyl ether at 40–60°C for 3 hours yielded only 13.5% pangus fish oil (Rahman *et al.*, 2023). Recently, Ciftci and Cavdar (2025) applied the UAE method to extract fish oil from Atlantic Bonito waste, achieving a yield of 39.41%.

Variations in oil yield across different studies may be influenced by raw material char-acteristics and extraction parameters. Biological factors—such as fish species, lipid distribu-tion within tissues, moisture content, and nutritional status-significantly affect extraction effi-ciency (Kuepethkaew *et al.*, 2025). Furthermore, extraction parameters including solvent-to-solid ratio, time, and temperature are critical for lipid recovery. A higher solvent ratio enhanc-es solvent diffusion into the sample, thereby increasing lipid solubilization and overall oil yield (Zhang *et al.*, 2018).

Table 4 Results of statistical analysis for quadratic model

Process	Std. dev	Mean	CV (%)	R^2	Adjusted R^2	Predicted R^2	Adeq. precision
Reflux	2.79	36.42	7.67	0.94	0.89	0.61	10.40
UAE	2.39	39.66	6.03	0.95	0.92	0.75	13.22

Table 4 Results of statistical analysis for quadratic model

Process	Std. dev	Mean	CV (%)	R^2	Adjusted R^2	Predicted R^2	Adeq. precision
Reflux	2.79	36.42	7.67	0.94	0.89	0.61	10.40
UAE	2.39	39.66	6.03	0.95	0.92	0.75	13.22

The higher oil yield obtained via the UAE method is likely due to the efficiency of acoustic cavitation, which increases the degree of cell wall disruption and subsequent lipid release (Shen *et al.*, 2023). UAE has been widely reported to enhance lipid recovery by dis-rupting cellular structures and improving solvent penetration into the biological matrix. This mechanism accelerates mass transfer and facilitates the release of intracellular lipids (Chemat *et al.*, 2017; Singla & Sit, 2021; Oprescu *et al.*, 2022).

Optimization of Reflux and UAE Extraction Process Conditions

The graphical representation of formula (7) and (8) and the optimization contour plots are shown in Figure 2 and 3 where the independent variables (solvent to solid ratio, and reaction time) affect the oil yield.

Figure 2 shows models the reflux extraction process, while Figure 3 shows the UAE. The optimal response point identified in this study is located at the midpoint (or center) of the tested range (Figure 1 and 2). In RSM, the midpoint is an important reference point in experimental design, offering preliminary insight into the behavior of the optimized model (Dharmegowda *et al.*, 2023). Both extraction methods demonstrated a positive relationship between reaction time and oil yield, though for different underlying reasons. In the reflux method, oil yield increases with longer extraction times due to the time required to break the raw material tissue structure (Purnamayati *et al.*, 2023).

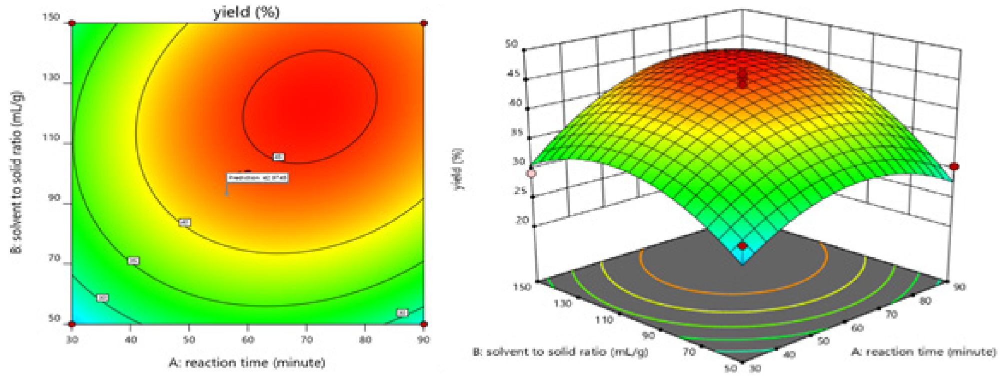

Figure 2 Contour plot extraction optimization of reflux method

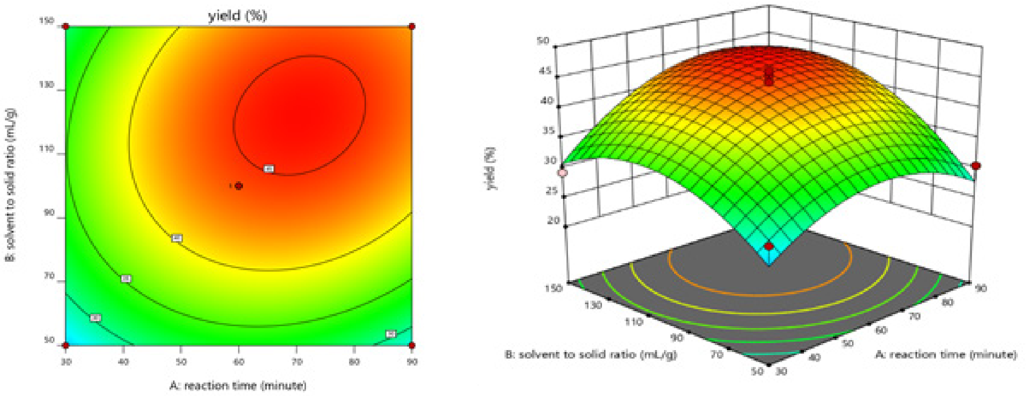

Figure 3 Contour plot extraction optimization of UAE method

In contrast, during the UAE extraction process, extending processing time leads to an increase in temperature which enhances the cavitation effect and facilitates the release of oil trapped in the raw material. Overall, the UAE method yields more oil in a shorter time and with less solvent usage compared with reflux extraction. These findings demonstrate that both reaction time and solvent-to-solids ratio significantly influence eel oil yield. The quadratic models for both the reflux and UAE extraction processes show that oil yield initially increases, then decreases as these variables continue to rise. This decline may result from saturation in the extraction system, where further increases in time or solvent are no longer efficient (Zhang *et al.*, 2018). Saturation occurs when most or all of the extractable oil has already been re-leased from the raw material. This point can be influenced factors such as the oxidation of bi-oactive compounds, reduced solvent efficiency, or the extraction process reaching its physical or chemical extraction limits.

Optimum Conditions of Reflux and UAE Extraction Process

The optimum conditions identified were used as a reference for conducting laboratory-scale eel oil extraction experiments using both reflux and UAE methods. The optimization process uses a desirability function, ranging from 0 (not desirable) to 1 (fully desirable), to measure how well the chosen conditions meet the set goals for each response variable (Solomando *et al.*, 2023). The optimum conditions of the two extraction processes with three repetitions with the quadratic model are presented in Table 5.

The predicted optimum values obtained were 45.87% (reflux) and 47.53% (UAE). Three replications of extraction showed that the UAE method produced a higher oil yield (47.7±0.03%) compared to the reflux (45.6±0.018%) while also requiring less extraction time and a lower solvent volume (Table 5). The UAE method has reported in literatures as a more effective method for increasing oil yield due to its effectiveness at extracting bioactive compounds (Shen *et al.*, 2023). Therefore, using the UAE method to extract fish oil reducing ex-traction time and solvent as well as increasing the release of metabolite compounds from the sample (Ivanovs & Blumberga, 2017).

Characterization of Eel Oil Quality

The eel oil produced from both extraction processes was tested for chemical quality. The test results with several important parameters can be seen in Table 6. The quality of eel oil (Table 6) indicates that while the reflux acid value (0.26 mg KOH/g) was lower than the UAE process (0.30 mg KOH/g) both are far below the International Fish Oil Standards. A low acid number indicates that the eel oil produced is of a high quality, while a high acid number indicates lower quality due to oil deterioration caused by hydrolysis of ester bonds and oxidation of double bonds (Nor *et al.*, 2021). Peroxide value in fish oil produced from both processes have also met the standards.

Table 5 Optimum conditions of eel oil extraction in reflux and UAE processes

Extraction process	Optimum condition		Oil yield (%)			Difference in response value	Desirability level	Accuracy (%)
	Time (min)	Ratio (mL/g)	PredictionDX$_{12}$	Experiment	Average			
Reaction time (min)	70.56	12.21:1	45.87	45.8	45.6±0.018	0.11	0.99	99.76
	70.56	12.21:1		48.0				
	70.56	12.21:1		43.0				
Solvent to solid ratio (mL/g)	69.50	11.21:1	47.53	52.5	47.7±0.03	0.17	1.00	99.64
	69.50	11.21:1		46.3				
	69.50	11.21:1		44.3				

Table 6 Quality characterization of eel oil

Testing	Extraction process		Standard (IFOS, 2015
	Reflux	UAE	
Acid value (milligrams of potassium hydroxide per gram (mgKOH/g))	0.26±0.06	0.30±0.03	≤2.25
Peroxide value (milliequivalents of active oxygen per kilogram (meq/kg))	4.70±0.30	4.80±1.00	≤3.75
Saponification value (milligrams of potassium hydroxide per gram (mgKOH/g))	158.95±16.1	166.43±3.23	180-256
Iodine value (gram iodine per 100 g of samples (gI2/100 g))	35.78±0.44	33.92±0.38	40-45
Free fatty acid (percent (percent))	1.33±0.34	1.47±0.29	≤1.5

Values are presented as mean ± standard deviation (from three replications).

Peroxide value (PV) is an indicator of oxidative degradation that shows the level of oil dam-age due to the formation of hydroperoxide compounds that give an unpleasant odor (rancidity). Lower peroxide value indicates higher quality of the oil (Nazir *et al.*, 2017). The PV obtained (4.70–4.80 meq/kg) is within the range commonly reported for freshly extracted fish oils, although it is slightly higher than the IFOS recommended limit of ≤3.75 meq/kg. This might be caused by lipid oxidation occurred during the initial extraction process. This elevation may be attributed to lipid oxidation occurring during the initial extraction stages. Specifically, drying the eel fillets prior to extraction may increase the contact between lipids and pro-oxidants, while exposure to heat and oxygen facilitates early oxidation.

Fish oils are highly susceptible to oxidation due to their high content of unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs) (Rozi, 2019). Exposure to oxygen, light, and elevated temperatures during extraction or solvent evaporation accelerates the formation of primary oxidation products, such as hydroperoxides, which are measured as PV (Ayu *et al.*, 2019; Dewita *et al.*, 2020). Therefore, the slightly elevated PV in this study likely reflects the inherent sensitivity of eel oil to processing conditions rather than a deterioration in oil quality.

Saponification values were also found to be within the recommended range. The saponification value refers to the amount of alkaline compounds required for the saponification reac-tion, and is determined by the molecular weight of the compounds contained in the fish oil (Ajikumar *et al.*, 2025). Other factors affecting the saponification value are fatty acid compo-sition and presence of triglyceride compounds. The higher the molecular weight of the com-pounds contained in the fish oil, the lower the saponification value (Ayeloja *et al.*, 2024a). Low saponification value also indicates a decrease in oil volatility which implies a flexible sapplication in the food industries (Kawekwune *et al.*, 2023). Fat and oil, which are both dom-inated by saturated fatty acid, tend to be more easily saponified than unsaturated fatty acids (Cheikhyoussef *et al.*, 2020). In general, eel oil contains various types of fatty acids with high-er unsaturated fatty acids than saturated fatty acids (Rahman *et al.*, 2023).

Fish oil with a high content of unsaturated fatty acids will bind large amounts of iodine, so iodine value is often used to measure the level of unsaturation in oils and fats. Iodine value is also determined by the degree of susceptibility to oxidative reactions (Krumreich *et al.*, 2024). The values from each extraction process were below the International Fish Oil Stand-ard (IFOS) (Table 6). This parameter is the quality standards that most often used to assess the quality of fish oil internationally. Oils with lower levels of unsaturation are less prone to oxi-dative rancidity (Purnamayati *et al.*, 2023). The extraction process affects the iodine value of eel oil (Ngamga *et al.*, 2024). In general, extraction methods that use solvents and heating af-fect the composition of fish oil thereby accelerating the oxidation of unsaturated fatty acids (Okalany *et al.*, 2024). In addition, residual proteins or impurities in fish oil cause changes in the double bond profile, thus reducing the iodine value (Arias *et al.*, 2022).

Free fatty acids (FFAs) are an important parameter for evaluating oil quality, as they provide a simple technique to monitor hydrolytic rancidity in fat-containing foods (Jayaprabakar *et al.*, 2024). Saturated fatty acids also contribute to the rancidity process in oils both directly and indirectly. FFAs are formed through triglyceride hydrolysis reactions that break fatty acid and glycerol bonds often as a result of the oxidation of double bonds. The oil quality decreases, the amount of FFAs increases leading to undesirable changes in taste and odor in fish oil (Arias *et al.*, 2022). Based on the analysis results, eel oil extracted using the reflux method met the standard limits for FFA's. The fatty acid composition of the fish oil influences the FFA content of a fish oil (Marques *et al.*, 2021). According to Budiadnyani *et al.* (2024), the highest percentage of fatty acid in eel oil is oleic acid, an unsaturated fatty acid with a single double bond (MUFA). MUFAs like oleic acid are more stable than polyunsatu-rated fatty acids (PUFAs) with multiple double bonds (Budiadnyani *et al.*, 2024). Greater numbers of double bonds contained in the fatty acid composition, lead to higher reactivity to oxygen so that the oil is more susceptible to oxidation and rancidity (Dongmo *et al.*, 2023).

Moisture Content of Eel Oil from Reflux and UAE Extraction Processes

Moisture content is a critical quality standard for crude fish oil. The presence of water compromises physicochemical stability and accelerates hydrolytic reactions during storage, which promotes the formation of free fatty acids (FFA) and leads to the deterioration of oil quality. Furthermore, moisture levels reflect the efficiency of extraction and post-extraction handling processes (Pang *et al.*, 2017; Gaydaybu *et al.*, 2020).

In this study, the moisture content of eel oil obtained from both extraction methods was notably low. Reflux extraction yielded a moisture content 0.02±0.03%, while the ultra-sound-assisted extraction (UAE) method resulted in a lower value of 0.01±0.01%. These results indicate that both techniques effectively minimize water content in the extracted oil. Fur-thermore, these values are significantly lower than the quality standards for fish oil, which are ≤1% according to the Indonesian National Standard (BSN, 2013) and 0.5–1% according to the International Fish Oil Standards (IFOS, 2015). Consequently, eel oil produced via reflux and UAE methods meets the recommended moisture standards for high-quality fish oil.

Moisture content of eel oil derived from each extraction process is below the specified quality standard value, while the water content in the eel oil from the reflux method is higher than from the UAE method. This is due to several factors including higher reaction temperatures, longer reaction times, and the use of more solvents than the UAE process (Zhu *et al.*, 2021). In contrast, the UAE extraction uses cavitation energy which lowers the reaction temperature, extraction time and solvent required leading to increased evaporation of water comes during evaporation process, and less moisture in the final product (Mehta *et al.*, 2022).

Moisture content plays a critical role in determining fish oil stability as the presence of water promotes hydrolytic reactions that cleave triglycerides into free fatty acids (FFA) (Aprilia *et al.*, 2021). Increased FFA content accelerates oxidative reactions by providing more active sites for oxygen interaction, thereby increasing the susceptibility of oil to lipid oxidation (Bruun *et al.*, 2021). Therefore, the lower moisture content observed in the UAE-derived eel oil contributes to improve oxidative stability compared to the reflux method. In addition, wa-ter is also a medium for the growth of microorganisms

(Djamaludin *et al.*, 2023) putting the oil at a higher risk of contamination which could result in the decrease of essential nutrients such as omega-3 and fat-soluble vitamins (vitamins A, D, E and K) (Zhai *et al.*, 2024). Furthermore, high moisture content limits the application of fish oil into food products (Donmez *et al.*, 2024) disturbed the stability of the product, and shorten its storage period.

Fatty Acid Composition in Eel Oil

Fish oil contains several types of fatty acids and other components such as sterols, vitamins, minerals, polyphenols and pigments. The fatty acids content is distinguished by the type of constituent chains such as saturated fatty acids, MUFA and PUFA (Awuchi *et al.*, 2022). The fatty acid composition of eel oil from each extraction method is presented in Table 7. Fatty acids variations can be attributed to differences in fat solubility, fatty acid chain length and the degree of saturation of the constituent compounds (Pandiangan *et al.*, 2021). From this it can be concluded that the extraction method significantly affects the quality and composition of fatty acids in fish

Table 7 Fatty acid profile of eel oil

Fatty acids	Chemical formula	% area (%)	
		Reflux	UAE
Lauric acid	C:12–0	0.1208	0.1366
Myristic acid	C:14–0	3.1094	3.2315
Pentadecanoic acid	C:15–0	0.2604	0.2499
Palmitic acid	C:16–0	22.9998	24.1278
Stearic acid	C:18–0	3.8699	4.2057
Arachidic acid	C:20–0	0.1505	0.1661
Heneicosanoic acid	C:21–0	0.6392	0.6299
SFA		31.150	32.7475
Palmitoleic acid	C:16–1	4.8374	4.6678
Cis-10-heptadecanoate	C:17–1	0.2703	0.2068
Oleic acid	C:18–1	45.1301	46.236
Eicosenoate acid	C:20–1	1.3601	1.2792
Erucic acid	C:22–1	0.6816	0.5461
MUFA		52.2795	52.9359
Linoleic acid **	C:18–2	12.5603	10.7214
Linolenic acid**	C:18–3γ	0.3841	0.3552
Linolenic acid*	C:18–3α	1.3791	1.1378
Eicosatrienoic acid*	C:20–3ω3	0.1402	0
Eicosatrienoic acid**	C:20–3ω6	0.5890	0.5694
Arachidonic acid**	C:20–4ω6	0.7477	0.7784
Eicosapentaenoic acid* (EPA)	C:20–5ω3	0.3002	0.2577
PUFA		16.1006	13.81
Omega 3*		1.8195	1.3955
Omega 6**		14.2811	12.4244

oil (Rahman *et al*., 2023). MUFA is the highest portion in both extraction processes. The reflux extraction process resulted in a higher PUFA content (18.34%) than UAE (16.01%). On the other hand, UAE extraction process produced slightly higher saturated fatty acid (SFA) and MUFA than reflux (Table 7). Palmitic acid is the most abundant saturated fatty acid and PUFA are reported to be beneficial for health (Ayeloja *et al*., 2024b).

The quality and composition of eel oil is influenced by many factors, both before during and after the extraction process. Eel oil is known to be a major source of omega-3 and omega-6. A previous study found that eel oil from West Java, contained omega-6 (8.24% w/w) and omega-3 (6.08% w/w) (Rawendra *et al*., 2021). This finding is in contrasts with the results of this research which obtained 14.28% omega-6 FA (reflux) and 12.42% from UAE, where the omega-6 FA is higher than that shown in previous studies and the omega-3 FA is lower, 1.82% from reflux and 1.39% from UAE. These differences are perhaps due to the habitat of the eels tested, including climatological conditions, feed sources, temperature and water quality which create a distinct nutritional profile (Sumi *et al*., 2023; Lall & Kaushik, 2021).

Previous studies have demonstrated that eel oil composition is strongly influenced by the extraction procedure employed (Mendez & Concha, 2018). However, post-extraction factors particularly storage temperature and storage duration also play a critical role in determining the stability and overall food safety of fish oil products. Increased storage temperature and prolonged storage duration have been reported to accelerate fish oil degradation (Noutsa *et al*., 2024; Medina *et al*., 2024).

Increasing public awareness of the importance of consuming eel oil as a source of omega-3 fatty acids has implications for the demand for eel oil (Faoziyah & Issusilaningtyas, 2020). This oil can be used as a raw material for omega-3 supplementation which is beneficial for children's growth and development, improving cognitive function and memory ability in malnourished children (Sittiprapaporn *et al*., 2022). In addition, the risk of premature birth and other health problems for pregnant women and toddlers can be reduced by increasing the in-take of eel oil (Schacky, 2020). This study emphasizes the quality characterization of eel oil based on its chemical features. Therefore, further investigation is needed to improve the quali-ty of eel oil from different habitats using different extraction processes.

CONCLUSION

This study determined the optimal conditions for eel oil extraction using reflux and ul-trasound-assisted extraction methods, with the highest oil yield obtained at a solvent-to-solid ratio of 10:1 and an extraction time of 60 minutes. The reflux and ultrasound-assisted extrac-tion methods produced eel oil with distinct yield efficiency and chemical quality. UAE offers advantages in processing efficiency, while reflux extraction yields eel oil with superior fatty acid characteristics relevant to nutritional quality. These findings highlight the importance of selecting appropriate extraction methods to optimize the nutritional value of fish oil. The optimized utilization of eel oil as a source of bioactive lipids may contribute for dietary and functional food applications.

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