

Supercritical carbon dioxide extraction of oil from *Thunnus tonggol* head by optimization of process parameters using response surface methodology

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Abstract—Total oil was extracted from ground fish head of Longtail tuna (*Thunnus tonggol*) using supercritical carbon dioxide (SC-CO₂) at 20 to 40 MPa, 45 to 65 °C and 1 to 3 ml min⁻¹. Response surface methodology (RSM) was employed to optimize the operating conditions of the SC-CO₂ technique where the highest oil yield was obtained (35.6% on dry weight basis) at 40 MPa, 65 °C, and 3 ml min⁻¹. The solubility of the oil in SC-CO₂ increased from 2.9 to 14.2 g oil/100 g of CO₂ with increasing pressure and temperature. The total saturated, monounsaturated and polyunsaturated fatty acids obtained were 41.6, 24.7 and 26.8%, respectively, where the omega-3 fatty acids were found to be 22.3%. A correlation was developed determining the coefficients of the second-order polynomial equation where the extraction parameters of SC-CO₂ method to extract fish oil from fish sample were successfully optimized using response surface methodology.

Key words: Fish Wastes, *Thunnus tonggol* Head Oil, Supercritical Fluid Extraction, Central Composite Rotatable Design, Correlations

INTRODUCTION

Global production of fish and shellfish is approximately 100 million tons and above 70% of this is processed [1], which mainly includes canning, filleting, smoking, and salting. As a result, the fish processing industry creates huge quantities of by-products including heads, skin, viscera, cut-offs and bones. Tuna heads, a non-edible by-product, are generated in large quantities (approximately 24% of total body weight) from the fish processing industry. Tuna is an important economic fish species in Malaysia and more than 90% of the tuna in Malaysia is *neritic* species, where long-tail tuna comprises about 50% of the total harvested tuna [2]. Among other species, long-tail tuna have become the main target species because of their high demand in the canneries of Malaysia and neighbor country, Thailand.

Fish by-products are good sources of oil ranging from 1.4 to 40.1% of the weight depending on the species and organ [3]. Saito et al. [4] extracted 19.9% lipid content from orbital organ of *T. tonggol* using Folch method. Docosahexaenoic acid (DHA), an omega-3 fatty acid, was characterized as the major fatty acid of all the highly migratory tuna fish, which accounts for more than 20% of the total fatty acids [4]. Separation of crude oil from skipjack tuna heads by wet-reduction method on non-precooked samples yielded 4.8% crude oil [5]. The benefit of fish oil containing omega-3 polyunsaturated fatty acids (PUFA) for human health has been extensively described in the literature [6]. Utilizing fish by-products is a good opportunity for production of fish oil rich in omega-3 PUFA. Novel extraction

procedures that result in high yield and high quality extracted oil are essential for effectively utilizing fish by-products. The common methods currently used for fish oil recovery are wet reduction, which includes cooking, pressing and centrifugation [5,7]; low temperature solvent extraction [8]; enzymatic hydrolysis [9]; alkaline hydrolysis [10]; and supercritical fluid extraction [11]. The assessment and optimization of an extraction process seem to be essential for its industrial application. Recently, RSM has been successfully employed to optimize SC-CO₂ extraction of fats, oils, and other bioactive compounds from different plant and animal sources such as hemp seed [12]; *Passiflora* seed [13]; sea pomegranate seed [14]; buckthorn [15]; wheat germ [16], *Vetiveria zizanioides* essential oil [17]. However, no studies have been reported on optimizing SC-CO₂-extraction of oil from tuna head using RSM. Thus, the objective of this study was to determine the effect of SC-CO₂ pressure, temperature and flow rate on oil yield and to develop a model equation that can determine the optimum processing conditions for SC-CO₂-extraction of total oil from tuna head using RSM. Solubility of CO₂ was also determined. Coefficients of Linest correlation were determined and compared with regression coefficients of RSM for confirmation of optimization studies.

MATERIALS AND METHODS

1. Materials

Long-tail tuna (*Thunnus tonggol*) were obtained from the fish landing center of Batu Maung, Pulau Penang, Malaysia. The solvents used for supercritical fluid extraction (SFE) were 99.9% pure CO₂ obtained from MOX-LINED gases Sdn Bhd (P.J., Selangor, Malaysia) and absolute ethanol (99.7%) were provided by QR&C™

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(QREC (Asia) Sdn Bhd, Rawang, Malaysia). Purified helium as a carrier gas with a purity of 99.9% was also purchased from MOX-LINED gases Sdn Bhd. Other solvents used in this study were of analytical grade and obtained from QR^{EC}™ (Malaysia).

2. Sample Preparation

Fresh fish samples were placed in insulated icebox and transported to the laboratory of the Environmental Technology Department, School of Industrial Technology, Universiti Sains Malaysia within 30 minutes. Samples were immediately de-headed using a butcher's knife and washed with enough cold tap water. The samples of fish head were freeze dried (Model: LABCONCO, USA) at a drying temperature of -47 °C followed by vacuum treatment at 0.133 bar for three days. The dry samples were kept in desiccators until used. Dried heads were ground in a blender (Waring, Laboratory, USA) into particle sizes ranging from 0.2 to 0.5 mm by sieving (USA standard testing sieve, A.S.T.M.E-11, W.S Tyler, USA).

3. Proximate Study

Proximate composition of tuna head tissues was determined in triplicate. Moisture and ash content were determined according to the standard AOAC [18] methods no. #952.08, and #938.08, respectively. Total nitrogen in the homogenized tuna heads was determined by Kjeldahl digestion method [18]. Protein content was calculated as 6.25 times %N. The crude lipid content was determined by the Soxhlet method (18). The extraction was carried out in triplicate, where 5 g (dry basis) of head powder was extracted with 200 ml of n-hexane for 6 hr. After lipid extraction, solvent was removed at 60 °C by using a rotary evaporator (Büchi-Rotavapor, Switzerland) and then placed in an oven at 45 °C for 1 hr until constant weight and percent lipids were determined. Oils were stored at -70 °C under nitrogen until further analysis.

4. Oxidative Stability Analysis

Rancidity and oxidative stability analysis was conducted in triplicate for each sample of tuna head oil extracted by Soxhlet extraction. The percent free fatty acids (% FFA as oleic acid) and peroxide values (PV) were determined using AOCS official methods [19] # Ca 5a-40 and Cd 8b-90, respectively.

5. Experimental Design for Supercritical Extraction

Central composite rotatable design (CCRD) was employed for response surface optimization of the operating conditions of SC-CO₂ extraction used for the experiment. Three independent parameters, namely temperature, pressure, and flow rate, were considered. The levels of these parameters were selected based on our preliminary study. The studied parameters included temperature (X_1) from 45 to 65 °C, pressure (X_2) from 20 to 40 MPa, and CO₂ flow rate (X_3) between 1 and 3 ml min⁻¹. The coded and un-coded variables used in the experimental design are listed in Table 1. The experimental design was based on CCRD consisting of 20 experimental settings with 2 levels full factorial, 8 cube points, 6 axial points and 6 central points in cube, as shown in Table 3. All the experimental trials except for the center point were randomly performed. Experiments at the center point were performed to estimate the pure error.

The generalized second order polynomial regression model was assumed for predicting the Y variable. The model proposed for the response of Y fitted Eq. (1) as follows:

$$Y = \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_{ii} X_i^2 + \sum_{i < j=1}^3 A_{ij} X_i X_j + J \quad (1)$$

Table 1. Uncoded and coded levels of independent variables used in the RSM design

Levels of coded variable	Independent variables		
	Temperature, °C (X_1)	Pressure, MPa (X_2)	Flow rate, ml min ⁻¹ (X_3)
Low (-1)	45	20	1.0
Center (0)	55	30	2.0
High (+1)	65	40	3.0
Axial (-1.68)	38.2	13.2	0.3
Axial (+1.68)	71.8	46.8	3.7

Table 2. Proximate composition of *Thunnus tonggol* head tissue and oxidative stability parameter of total lipids

Proximate compositions	Value
Moisture (%)	69.9±1.6
Protein (%)	14.7±1.4
Lipid (g/100 g dry sample)	36.2±0.3
Ash (%)	4.5±0.4
Oxidative stability of head oil	
Peroxide value (meq O ₂ /kg)	2.2±0.3
Free fatty acid (% as oleic acid)	1.9±0.2

Table 3. Oil yield (% mean±SD) extracted from head of *Thunnus tonggol* using SC-CO₂ method against the matrixes of CCD of RSM

Run order	Temperature (°C)	Pressure (MPa)	Flow rate (ml min ⁻¹)	Oil yield (%)
1	45.0	40.0	3.0	34.2±0.3
2	55.0	30.0	2.0	27.6±0.2
3	45.0	40.0	1.0	22.1±0.5
4	55.0	30.0	2.0	30.1±0.3
5	38.2	30.0	2.0	22.0±0.2
6	55.0	30.0	3.7	35.1±0.5
7	71.8	30.0	2.0	24.9±0.2
8	65.0	20.0	1.0	13.3±0.4
9	55.0	30.0	2.0	30.1±0.3
10	55.0	30.0	2.0	30.2±0.2
11	45.0	20.0	1.0	8.3±0.1
12	45.0	20.0	3.0	24.7±0.2
13	55.0	30.0	2.0	30.3±0.5
14	65.0	40.0	1.0	27.8±0.4
15	55.0	30.0	0.3	18.1±0.3
16	65.0	40.0	3.0	35.6±0.2
17	55.0	13.2	2.0	4.5±0.2
18	55.0	46.8	2.0	35.3±0.5
19	65.0	20.0	3.0	14.7±0.3
20	55.0	30.0	2.0	30.1±0.7

where Y is the response (extracted oil yield, %) and A_0 , A_i , A_{ij} , and J are constant coefficients of linear, square, interaction, and intercept, respectively. X_i and X_j are independent variables (temperature, pressure, and CO₂ flow rate) (Eq. (1)). Three-dimensional response

surfaces plots were generated by changing two variables within the experimental range and keeping the other variable constant at the central point. The statistical software Minitab 14 (Minitab Inc, PA, USA) was used for the data of analysis of variance (ANOVA) and regression analysis (R^2). The regression coefficients were further determined for the 20 experimental settings using Linest correlations model for comparison and verification in the event that R^2 were found to be further low. However, the quality of the regression model was evaluated by the coefficient of determination (R^2), and the statistical significance of the model was based on 95% confidence level.

6. Supercritical Fluid Extraction (SFE)

The experimental set-up for the SC- CO_2 extraction process was assembled according to Norulaini et al. [20]. The ISCO SFE System (ISCO Inc., Lincoln, NE, USA) included a supercritical fluid extractor (ISCO, SFX 220), controller (ISCO, SFX 200), CO_2 cylinder, a chiller (Yih Der BL-730), two syringe pumps (ISCO, Model 100DX), CO_2 pump, and co-solvent pump. The temperature controller was associated with two coaxially heated capillary restrictors (ISCO). The CO_2 pump was fitted into a cooling jacket to deliver CO_2 , and the other pump to deliver co-solvent as modifier/entrainer. Pumps were kept cool with an ethylene glycol-deionized water solution (50 : 50, v/v) which was circulated through the cooling jacket using a refrigerated circulator. The temperature of the chiller was maintained at 0 °C. In each experiment, 2 g of ground sample (dry weight) was loaded into a 2 ml sample canister and placed in the ISCO extraction chamber and allowed to equilibrate at the desired temperature. The extractions were performed using CO_2 and ethanol (as a co-solvent) at a ratio of 80 to 20% (v/v). The total flow rates were set at 0.3, 1, 2, 3, and 3.7 ml min⁻¹ for 16, 5, 3, 2 and 1.8 hrs, respectively, where the temperatures ranged between 45 and 65 °C and the pressures from 20 to 40 MPa. At supercritical conditions, the dissolved oil was separated with SC- CO_2 and collected into a pre-weighed blue cap bottle and cooled with ice-water mixture. The bottle, which contained the extracted oil with ethanol as a residue co-solvent, was evaporated under vacuum at 40 °C using a rotary evaporator (Büchi-Rotavapor, Switzerland) and placed in oven at 40 °C for 30 min before being transferred into the desiccator. The extractability was expressed as percentage based on 100 g of ground dry sample as defined below:

$$\text{Extractability of oil yield (\%)} = \frac{\text{Total yield (g) extracted by SC-}\text{CO}_2}{\text{Total yield (g) extracted by Soxhlet extraction}} \times 100$$

7. Solubility and CO_2 Consumption

The formula and equation for the calculation of solubility and CO_2 consumption were based on our previous studies [21].

8. Fatty Acid Analysis

The fatty acid (FA) constituents were analyzed by gas chromatography with flame ionization detector (GC-2010 Plus AOC-5000, Shimadzu, Osaka, Japan). Fatty acid methyl ester (FAME) was prepared by dissolving 50 mg of sample into 0.95 ml n-hexane, and 0.05 ml 1 M sodium methoxide (30% methanol in sodium methoxide). The mixture was then shaken vigorously using an auto-vortexer (Janke and Kunkel, VF2, Germany) for 30 s and let to stand for a wait time of 5 min so that it formed a bilayer. The clear upper layer containing the FAMEs (1 μ l) was pipetted off and injected into a GC using standard method [19]. Supelco 37 component FAME

mixtures (Sigma-Aldrich, Supelco, Bellefonte, PA., USA) was used as reference standard fish oil (purity 99%). The BPX70 [70% Cyano-propyl polysilphenylene-siloxane (30 m \times 0.32 mm \times 0.25 μ m film thickness), SGE France] was purchased from Sigma-Aldrich Co., USA. The oven temperature was set at 140 °C, held for 2 min, increased with a heating rate of 5 °C/min up to the final temperature of 250 °C, and then held for 10 min at 250 °C. Chromatographic peaks were identified by using the retention time of FAME standard. Results of fatty acids analysis were reported as an average of three analyses for each sample investigated.

RESULTS AND DISCUSSION

1. Proximate Analyses and Characterization of Tuna Head Oil Yield

In proximate analyses, the total moisture content of the wet fish head was found to be 69.9% whereas the total protein, total lipid and ash content were found to be 14.7%, 36.2 g/100 g dry sample (that is equivalent to 10.9% wet basis) and 4.5%, respectively (Table 2). These results are in line with the report of Aidos et al. [22], who reported moisture, protein and lipid content of 66.9%, 14.3% and 129 (g/kg wet sample), respectively, from herring head by-products. Vlieg and Murray [23] studied the proximate composition of albacore tuna head and found moisture, protein, lipid and ash content were 61.2%, 19.5%, 13.6% and 5.7%, respectively. In this study, the total protein was found to be relatively low, and the reason may be that the fish heads are mainly composed of bones with much less muscle tissues. However, the purpose of determination of proximate composition was to determine the variation in the chemical compositions of the tuna head which is attributed to the species, maturity, dissected body parts and processing conditions. The peroxide values (PV) and free fatty acids (FFA) as determinations of oxidative stability of oil were obtained at 2.2 meq $\text{O}_2/\text{kg}^{-1}$ and 1.9% (Table 2). Bimbo [24] reported the acceptable limit of PV and FFA for crude fish oil is 3-20 meq O_2/Kg and 2-5%, respectively. The quality of fish oil deteriorates with longer storage time at high temperature [22]. Researchers reported that the oil process at relatively low temperature could have less PV and FFA, lowering the hydrolysis of ester bonds of triglycerides [5]. However, supercritical fluid extraction could be one of the recommended methods for low temperature extractions. The moisture content was further determined on the freeze dried and ground fish head before supercritical fluid extraction, which was found to be 2.7%. It has been reported that moisture levels between 3% and 15% had little or no effects on the extractability of oils from seed with SC- CO_2 [25]. Devittori et al. [26] reported that a moisture reduction from 8 to 1% had no effect the extraction and the oil mass transfer in SC- CO_2 . In this study, we did not observe any noticeable effect of moisture on the extraction of the materials studied.

2. Effect of SFE Parameters

The efficiency of SC- CO_2 oil extraction is generally dependent on pressure, temperature, flow rate and time. Temperature, pressure and flow rate showed a positive linear effect on the oil yield (Table 4). The quadratic term had a negative effect on the oil yield; in addition, the complex interaction between temperature and pressure, and pressure and flow rate showed a positive effect on the oil yield (Table 4).

Table 4. Regression coefficients of RSM (Coef-RSM) and coefficient of Linest Correlations (Coef-Linest), and R² for the final reduced models between Linest correlation and response surface

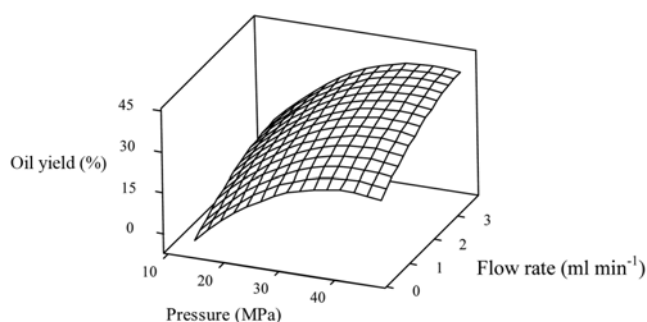
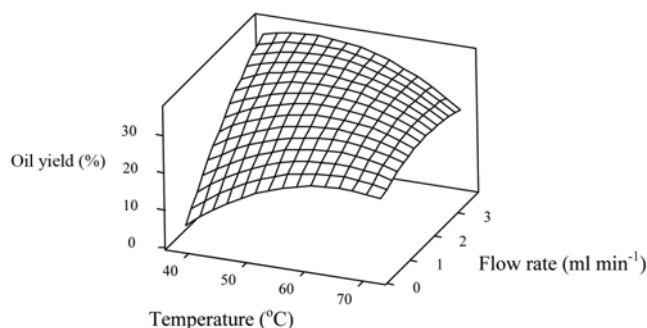
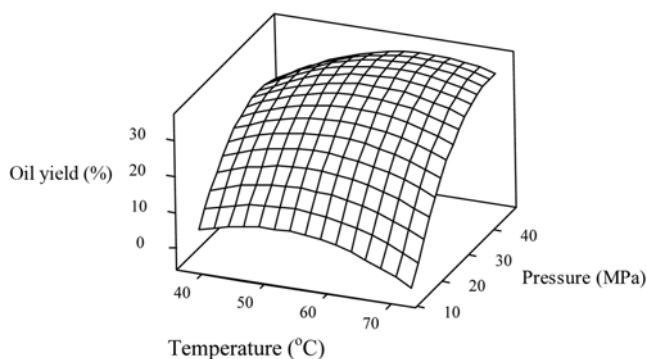
Term	Coefficient of linest	Coefficient of RSM	T	P
Const. (J)	-113.107	-113.077	-5.521	<0.001
T (A)	2.621	2.602	4.528	<0.001
P (B)	2.059	2.054	4.337	<0.001
F (C)	22.045	22.053	4.940	<0.001
T*T (D)	-0.023	-0.023	-4.813	<0.001
P*P (E)	-0.034	-0.035	-7.447	<0.001
F*F (F)	-1.181	-1.180	-2.475	<0.033
T*P (G)	0.015	0.015	2.365	<0.040
T*F (H)	-0.241	-0.241	-3.772	<0.004
P*F (I)	0.026	0.026	0.410	<0.690
R ² (%)	0.9790	0.9790		
Lack of fit (F-value)		4.96		
Lack of fit (P-value)		0.052		

At a given temperature and flow rate, the total oil yield increased with the pressure (Table 3; run order no. 1, 12; 3, 11; 8, 14; 16, 19). Lowest yield (4.5%) was found in the low pressure of 13.2 MPa and the yields increased with increasing pressure. This might be due to the increased solvent density resulting in increased oil solubility [14]. An increasing trend in the total oil yield with increasing flow rate was observed at a constant temperature and pressure (Table 3; run order no. 1, 3; 8, 19; 11, 12; 14, 16). Temperature had a negative effect on the recovery of the yield because the density of CO₂ decreased with increase in temperature, and that may be true at low pressure (Table 3; run order no. 12, 19). The yield increased with pressure and temperature when pressure was above 30 MPa. This phenomenon was reported for SC-CO₂ extraction of seed oils [14, 15] and found to be true in this study. At constant pressure (40 MPa) and flow rate (3 ml min⁻¹), the yield increased from 34.2 to 35.6% with increasing temperature from 45 to 65 °C, respectively (Table 3; run order no. 1, 16). Similar trend was observed in the run order no. 3, 14; 11, 8 (Table 3). However, the combined effect of temperature, pressure and flow rate was compensated by gain in the density of SC-CO₂, which means an increase in the solubility of oil in the solvent. Temelli et al. [27] reported that the impact of temperature is dependent on competing parameters; with increasing temperature, the density of CO₂ decreases while the vapor pressure of solutes increases, enhancing mass transfer kinetics during extraction. The statements are in agreement with our observations regarding the effect of pressure, temperature and flow rate on the recovery of oil as shown in Table 3.

The highest yield was obtained at the combined conditions of 40 MPa, 65 °C, and 3 ml min⁻¹. The yields were 35.6 g/100 g sample on dry basis by SC-CO₂ extraction. This yield was similar to the yield from the Soxhlet extraction method (36.2 g/100 g sample, on dry basis). In a separation process, the addition of modifier/co-solvent would be beneficial if the selectivity could be improved due to specific intermolecular interactions between the co-solvent and specific components of the mixture [27]. However, in this study,

the highest yield was obtained with the co-solvent or modifier; hence its use could be recommended to overcome the shortcoming of the existing conventional methods in terms of time saving, cost effectiveness, removal of toxic residues as a result of achieving green, environmental friendly and safe products. Moreover, ethanol can be easily evaporated, with or without vacuum, to acceptable levels in terms of oil quality.

The best way to visualize the effects of SC-CO₂ parameter on oil yield is by drawing response surface plots based on regression equation. The response surfaces plots that were developed using the fitted second order polynomial equation are presented in Figs. 1, 2 and 3 as a function of the two variables by holding the third one

**Fig. 1. The generated response surfaces plots for the oil yield (Y) as a function of pressure (X₂) and flow rate (X₃), at a fixed temperature of 55 °C for SC-CO₂ extraction.****Fig. 2. The generated response surfaces plots for the oil yield (Y) as a function of temperature (X₁) and flow rate (X₃) at a fixed pressure of 30 MPa for SC-CO₂ extraction.****Fig. 3. The generated response surfaces plots for the oil yield (Y) as a function of temperature (X₁) and pressure (X₂) at a fixed flow rate of 2.0 ml min⁻¹ for SC-CO₂ extraction.**

constant.

Predicted response surface for oil yield as a function of pressure and CO₂ flow rate is presented in Fig. 1. At 65 °C, oil yield increased from 4.5% to 35.6% with increasing pressure from 20 to 40 MPa and flow rate from 1 to 3 ml min⁻¹. Fig. 2 shows the effect of temperature and flow rate on the yield at a fixed pressure of 30 MPa for SC-CO₂ extraction. Maximum oil yield was achieved at minimum consumption of solvent at 3 ml min⁻¹. Our results are consistent with the study of SC-CO₂ extraction of seed oil [16]. When the flow rate of CO₂ increased, extracted yield of oil increased at first, but began to decrease when the CO₂ flow rate surpassed 3 ml min⁻¹. Decrease in total oil yield at high flow rate might be either by an elevated pressure drop through the extraction cell or increasing analyte loss during decompression of the fluid [28]. Fig. 3 shows the surface plot of tuna head oil yield as a function of temperature and pressure at a fixed flow rate of 3.0 ml/min. The oil yield increased with pressure up to 40 MPa and the highest amount was obtained at 65 °C and 3.0 ml/min. The solubility of fish oil depends largely on the balance between fluid density, solute vapor pressure, and the pressure which was predominant in this situation [20,28].

3. Fitting the Models

Table 3 shows the oil yield obtained from the experimental set according to RSM design. The experimental data were analyzed by ANOVA to assess the accuracy of fit in Table 4. Table 4 also summarizes the regression coefficients of RSM, coefficient of Linest correlations, R² and probability values for the final reduced models. ANOVA (*F*-test) and *p*-values were used to check the significance of each coefficient and that indicated the interaction strength of each parameter [28]. In our study, a *p*-value of <0.05 meant that the model was statistically significant for both techniques, which indicated the model was suitable for our experiments. *F*-value and *p*-value for the lack of fit of the model was 4.96 and 0.052, which was non-significant (*p*>0.05), confirming the validity of model. The coefficient of determination (R²) was 0.97901 and 0.9790 for the Linest and RSM, respectively (Table 4), revealing that the polynomial model adequately represents the experimental results. The second-order polynomial model as a function of independent variables for the oil yield (*Y*) is given below:

$$Y = AX_1 + BX_2 + CX_3 + DX_1^2 + EX_2^2 + FX_3^2 + GX_1X_2 + HX_1X_3 + IX_2X_3 + J \quad (2)$$

From Table 4 and Eq. (2), the significant positive effects on the oil yield were due to the linear terms of flow rate (*X*₃) (*p*=0.001), pressure (*X*₂) (*p*=0.001) and temperature (*X*₁) (*p*=0.001). In the quadratic terms, temperature (*X*₁²) (*p*=0.001), pressure (*X*₂²) (*p*<0.001) and flow rate (*X*₃) (*p*=0.033) had significant negative effects on the yield. However, the terms of interactions between the temperature and pressure (*X*₁*X*₂) (*p*=0.040) and temperature and flow rate (*X*₁*X*₃) (*p*=0.004) had significant positive and negative effects on the oil yield. Therefore, based on the above models obtained using CCRD of RSM where the correlations were developed using the Linest correlation models, we observed that the optimal conditions for tuna head oil yield were 40.0 MPa pressure, 65 °C temperature, and 3.0 ml min⁻¹ CO₂ flow rate, at which the highest yields were obtained (run order no. 16) (Table 3).

4. Influence of Co-solvent to the Solubility of Fish Oil in SC-CO₂

Table 5 shows the solubility and total amount of CO₂ required to

Table 5. The solubility and total CO₂ used for the SC-CO₂ extraction of oil from tuna head at different pressures, flow rate and temperature

Run order	Temperature (°C)	Pressure (MPa)	Flow rate (ml min ⁻¹)	CO ₂ used (g)	Solubility (%)
1	45.0	40.0	3.0	272.7	12.5±0.2
2	55.0	30.0	2.0	261.2	10.6±0.3
3	45.0	40.0	1.0	227.3	9.7±0.1
4	55.0	30.0	2.0	261.2	11.5±0.4
5	38.2	30.0	2.0	270.4	8.1±0.1
6	55.0	30.0	3.7	293.9	11.9±0.2
7	71.8	30.0	2.0	243.9	10.2±0.1
8	65.0	20.0	1.0	199.0	6.7±0.2
9	55.0	30.0	2.0	261.2	11.6±0.1
10	55.0	30.0	2.0	261.2	11.5±0.3
11	45.0	20.0	1.0	214.3	3.9±0.3
12	45.0	20.0	3.0	257.2	9.6±0.1
13	55.0	30.0	2.0	261.2	11.6±0.6
14	65.0	40.0	1.0	215.5	12.9±0.2
15	55.0	30.0	0.3	261.2	6.9±0.2
16	65.0	40.0	3.0	258.6	13.8±0.3
17	55.0	13.2	2.0	240.5	1.9±0.1
18	55.0	46.8	2.0	269.0	13.1±0.4
19	65.0	20.0	3.0	238.8	6.2±0.2
20	55.0	30.0	2.0	261.2	11.5±0.1

extract the tuna head oil. The total amount of CO₂ consumed increased with pressure and decreased with temperature, but the combined effect of higher pressures and temperatures resulted in exponential increase in the density and solubility due to a similar increase in vapor pressure of the oil [29]. As the density increased, the interaction between oil and CO₂ also increased, thus leading to higher mass transfer rates and property changes [30]. Higher yields using lower amounts of CO₂ are economically beneficial and provide an optimal final product for human consumption [31].

According to Table 5, it became evident that the addition of ethanol as co-solvent could greatly enhance the solubility of SC-CO₂ in fatty acid constituents of fish oil. Ethanol concentration significantly (*P*<0.05) increased the solubility of fish oil or increased the extraction efficiency notably. In general, extraction yield increases with pressure due to an increase in lipid solubility in SC-CO₂ based on an increase in CO₂ density [27]. The diffusivity of fish oil was increased in the presence of ethanol that also increased the solvating power of SC-CO₂. In other words, dissolving fish sample in ethanol increased the potential solubility of triglycerides; thus higher oil yield were obtained (Table 5). The solubility of the oil in SC-CO₂ increased from 1.9 to 13.8 g oil/100 g of CO₂ as the pressure, temperature and flow rate were increased from 20 to 40 MPa, 45 to 65 °C and 1 to 3 ml min⁻¹, respectively (Table 5). Dobbs et al. [32] reported that addition of co-solvent in SC-CO₂ may facilitate separation of solutes that have various polarities. This may be one of the reasons for getting higher yield in co-solvent assisted techniques compared to applying SC-CO₂ alone in a continuous process (Table 3). Thus, solubility enhancement is the result of an increase in the density of the SC-CO₂+co-solvent mixture or inter-

molecular interactions between the co-solvent and a particular solute [27].

Sánchez-Camargo et al. [33] extracted shrimp wastes oil and reported that the addition of ethanol with SC-CO₂ significantly increased the yields to 136% when the proportion of ethanol was increased from 5% to 15% wt. In the case of liquid CO₂ extractions, solubility was almost twice that obtained with no entrainer [33]. Similar finding were reported for determining the solubility of sunflower oil in CO₂, by Cocero and Calvo [34]. The authors reported that oil solubility was increased at the rate 5 g/kg CO₂ for each percent (by weight) addition of ethanol in SC-CO₂ at 30 MPa and 42 °C. The results obtained here are also in line with Olimpio et al. [35], who reported that the solvent power of SC-CO₂ increased with adding even a little amount of co-solvent, and the effect depended on the concentration of the co-solvent at supercritical state and it could be explained by the phase behavior of ethanol and SC-CO₂ mixtures at various operating parameter especially temperature and pressure. The effect of ethanol that could be denoted as polar co-solvent that has potential extractability due to the affinity of ethanol to the sample matrixes facilitating the adsorption ability [36].

Arul et al. [37] also reported that the solubility of triglycerides, in terms of fatty acids constituents, in SC-CO₂ could be influenced by extraction conditions and solubility trends to reach saturation level, temperature, and pressure could be the most considerable factors for dissolving the solutes in supercritical fluids [38,39]. In addition to pressure and temperature other factors influencing the solubility and yield are the length of the hydrocarbon chain and the presence of functional groups [40]. Therefore, short-medium chain and saturated fatty acids can be extracted in shorter time in comparison to relatively longer chain, unsaturated and molecules with higher molecular weight. Hydrogen bonding, in the presence of a co-solvent like ethanol or tributyl phosphate, is one of the influencing factors for enhancing the solubility of some solutes in supercritical fluids [32,41].

Table 6. Fatty acid composition of *Thunnus tonggol* head wastes oil extracted by SC-CO₂ (values are mean±SD; n=3)

FA	Amount (%)
C14:0	6.3±0.1
C15:0	1.5±0.2
C16:0	25.1±0.3
C17:0	1.1±0.1
C18:0	7.8±0.1
C16:1n7	4.1±0.1
C18:1n9	15.4±0.2
C18:1n7	2.4±0.1
C20:1n9	1.4±0.2
C22:1n11	1.5±0.3
C18:2n6	1.1±0.3
C18:4n3	1.2±0.2
C20:4n6	2.6±0.1
C20:5n3	1.5±0.1
C22:6n3	19.7±0.3
Total SFA	41.6
Total MUFA	24.7
Total PUFA	26.8

5. Fatty Acid Constituents in Tuna Head Oil

Table 6 shows the fatty acid constituents of tuna head by-product oil extracted by SC-CO₂ at 45 °C, pressure 40 MPa and flow rate 3 ml min⁻¹. The total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and PUFAs were found to be 41.6, 24.7 and 26.8%, respectively. Among PUFAs omega-3 fatty acids were found to be 22.3% (1.1% C18:4n3, 1.5% C:5n3, 19.7% C22:6n3) among all other fatty acid constituents in the tuna head oil extracted using co-solvent with SC-CO₂ extraction. C16:0 (25.1%) was the highest among the SFAs whereas, C18:1n9 (15.4%) was found to be highest among the MUFAs. The differences among SFA, MUFA and PUFA contents in the head oil of tuna were significant.

Chantachum et al. [5] reported the range of 47.0 to 51.0%, 21.1 to 25.0% and 24.0 to 31.6% of SFAs, MUFAs and PUFAs constituents, respectively, in the oil were extracted from both precooked and non-precooked tuna head at 85 °C for 30 min. The researchers also reported the range of fatty acid constituents of 27.9 to 30.3% of C16:0, 14.1 to 16.5% of C18:1 and 18.8 to 25.5% of C22:6. Our observations were in line with the observation of Chantachum et al. [5]. Intarasirisawat et al. [42] extracted total lipids from tuna roes and reported C18:1 n-9 constituent as major MUFA whereas, the C16:1 n-7 and C18:1 n-7 as the n-7 fatty acids which were found to be low in content of fatty acid constituents. This is because of n-7 fatty acids especially C16:1n7 and C18:1n7 are regarded as ubiquitous and minor components in the animal tissue [42]. Our observations are also similar with the reports of Saito et al. [4] where the authors reported that the head (orbital) part contains the highest amount of total PUFA than any other tuna wastes. The trend of neither PUFA nor MUFA was found in the total amount of SFA content which was relatively high in the head part.

CONCLUSION

The use of ethanol as a co-solvent with SC-CO₂ was very effective in recovering almost all of the oil from longtail tuna (*Thunnus tonggol*) head with least amount of CO₂ consumption. The method was successfully optimized at 40 MPa, 65 °C, and 3 ml min⁻¹ where the highest solubility (13.8%) of the fish head oil was found in SC-CO₂. A multiple regression equation was also obtained by using results from 20 experimental settings in CCRD and applying RSM. A correlation was developed using LINEST where the coefficients of the variables (extraction parameters) were found to be much closer to the coefficients of the RSM. Finally, a new correlation was successfully developed where the reduction in coefficient (R²=0.9790) predicted the effects of extraction parameters on oil yield in SC-CO₂ extraction method. However, omega-3 fatty acids existed at 22.3% in 26.8% of total PUFAs. Further study could be conducted for the simultaneous extraction and fractionation of fatty acids, especially omega-3 rich fish oil from fish by-products using co-solvent method of SC-CO₂ extraction at the above mentioned optimized condition.

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