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Effectiveness of Urea Crystallization Method in the Producting Omega-3 Concentrate from Tuna (*Thunnus* sp.)

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ABSTRACT

Omega-3 concentrates developed from fish oil without any other components are excellent for overall health. The objective of this study was to evaluate the effectiveness of the urea crystallization method in relation to the iodine value and the increase in total omega-3 fatty acids during the production of omega-3 concentrates from tuna fish oil using response surface methodology (RSM). Using the urea crystallization method, pure ethyl ester oil is mixed with urea, then left to stand at a low temperature with predetermined ratios and times to obtain omega-3 concentrate. The total omega-3 content in the crude tuna oil obtained in this study was 19.51%. The total omega-3 content from pure tuna oil was 32.90%. Meanwhile, the total omega-3 content in the tuna omega-3 concentrate was 16.97%. Longer storage times were found to reduce saturated fat content and increase omega-3 levels, as indicated by higher iodine values.

Keywords: low-temperature crystallization, omega-3 concentrate, response surface methodology. tuna oil

INTRODUCTION

Fish oil is a popular supplement due to its high content of omega-3 fatty acids. These fatty acids include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The carbon chain of DHA is structurally longer than that of EPA. This difference results in distinct characteristics and metabolic effects on human health between DHA and EPA. In particular, DHA is beneficial for fetal brain development during pregnancy, maintains retinal health, and helps prevent cardiovascular disease (Patel *et al.* 2022). Tuna fish oil is reported to have the highest DHA content at 27.33% compared to other fish species (Apituley *et al.* 2020). According to the findings of Suseno *et al.* (2014), tuna fish oil (*Thunnus sp*.) has the highest DHA content at 24.56%.

Fish oil produced in Indonesia is generally a byproduct of the canning, surimi, and fish meal industries. However, the fish oil available in the Indonesian market is pure fish oil with a relatively high saturated fat content. In fact, excessive consumption of saturated fatty acids contributes to an increase in diabetes and obesity (Islam *et al.* 2019). On the other hand, demand for fish oil in Indonesia continues to rise due to growing public awareness of the health benefits of fish oil. Fish oil import data in Indonesia has increased nearly tenfold from 2019 to 2021 (UN Commodity Trade Statistics 2022). One approach to addressing this issue is to produce omega-3 concentrates derived from pure fish oil. Omega-3 concentrates are considered better for health because they have been shown to possess anti-inflammatory activity, making them effective at reducing inflammation (Giacobbe *et al.* 2020).

One method developed for producing omega-3 concentrates is urea crystallization. This method was chosen because it does not require high temperatures and the investment costs for its implementation are relatively low. The urea crystallization method had previously been tested in a study by Estiasih (2010) regarding the production of omega-3 concentrate from lemuru fish oil, which showed a 2.61-fold increase in total EPA and DHA using a urea-to-fatty acid ratio of 2.9:1. Therefore, this study evaluated urea crystallization methods based on iodine value parameters and the increase in total omega-3 fatty acids, optimized using response surface methodology (RSM), to provide an overview of the fatty acid profile in omega-3 concentrates derived from tuna, as well as to provide information regarding the most effective time and conditions for producing omega-3 concentrates.

METHODS

Characterization of crude tuna oil

The primary material used was tuna oil from fish meal production at UD Samudra Kencana, Jembrana Regency, Bali. The study was conducted from April to October 2025. Crude oil characterization was performed to assess the quality of the fish oil prior to

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further use. At this stage, analyses were performed for total oxidation (TOTOX), free fatty acid (FFA) content, acid value (AV) using the AOCS (1998) method, peroxide value (PV), p-anisidine value (AnV), and iodine value using the Wijis method (AOAC 2005), and fatty acid profiling using gas chromatography (GC).

Purification of crude tuna oil

This stage involves bleaching the crude tuna oil mixed with 5% (w/w) Megnesol XL adsorbent for 20 minutes at a temperature of 50°C. This is followed by decantation for 24 hours and filtration until semi-purified fish oil is obtained (Suseno *et al* 2021).

Transesterification of tuna fish oil

Transesterification is carried out using NaOH as a catalyst. The ratio of semi-purified fish oil to NaOH solution is 6:1 (w/w) for 75 minutes at a temperature of 55°C. The product resulting from this process is a layer that is subsequently evaporated until pure ethyl ester oil is obtained.

Preparation of omega-3 concentrate

The preparation of omega-3 concentrate was carried out using urea crystallization (Estiasih *et al.* 2009, Chamidah and Wicaksono 2021). The urea solution was mixed with ethyl ester oil at a (v/v) ratio adjusted for each treatment, namely A (1.15:1), B (1.4:1), C (2:1), D (2.6:1), and E (2.85:1). The mixture was stirred for 30 minutes, then left to stand for some time until liquid and solid fractions formed. There were differences in treatment times, including 16 hours 48 minutes, 19 hours 18 minutes, 25 hours 18 minutes, 31 hours 18 minutes, and 33 hours 47 minutes, as determined based on the Response Surface Methodology (RSM) calculation. The resulting liquid fraction was then processed to obtain an omega-3 concentrate. Subsequently, the iodine value and yield of the omega-3 concentrate were analyzed. The sample with the highest iodine value was then further analyzed for its fatty acid profile.

Data Analysis

The optimization of omega-3 concentrate production from tuna in this study utilized 13 formulation combinations with two independent variables: the urea-to-fatty acid ethyl ester ratio (Variable A) and storage duration (Variable B). The iodine value and yield were the response variables observed. The determination of optimal conditions in this study utilized Design Expert software version 13 (DX13). RSM was employed to analyze the relationship between process variables and responses. Optimizing independent variables in a process enables the identification of optimal operating conditions, thereby enhancing both the quality and efficiency of the process.

RESULTS AND DISCUSSION

Characteristics of crude tuna oil

The characteristic values of crude tuna oil obtained were compared with the quality standards established by the Codex Alimentarius Commission (CAC) in 2021. Based on the data in Table 1, it is evident that most of the parameters of crude tuna fish oil meet the International Fish Oil Standard (IFOS) (2014). However, the peroxide value (PV) exceeds the standard. This is likely due to the raw material used being a byproduct of the canned fish industry. It is known that this industry typically involves high temperatures during the production process. The use of high temperatures during this production process triggers faster oxidation, thereby affecting the free fatty acid (FFA) parameter value in this study. The FFA value reflects the degree of fatty acid degradation in the raw material due to hydrolysis and oxidation reactions (Homayooni *et al.* 2014). The total oxidation (TOTOX) parameter value remains within acceptable limits, indicating that the oxidation process during production is still under control and the quality of the crude tuna oil has not yet experienced a significant decline.

The analysis of the fatty acid profile of crude tuna oil aims to identify the fatty acid composition, which includes saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), thereby revealing the dominant types of fatty acids in crude tuna oil. Based on the results presented in Table 2, it is known that the crude tuna oil samples contain 24 types of fatty acids. The fatty acid composition is dominated by palmitic acid, which is classified as a saturated fatty acid (SFA), at 13.81%. These findings align with other studies showing a palmitic acid content of 13.88% in tuna oil (Suseno *et al.*, 2014). Differences in fatty acid levels across various studies are likely due to oxidation occurring during storage (Suriani *et al.*, 2014).

Purification of crude tuna oil

The purification of crude tuna oil through a bleaching process aims to reduce the undesirable components contained in the fish oil (Apituley 2020).

Table 1 Oxidation parameters of raw tuna oil

Parameter	Crude Oil Analysis Results	IFOS (2014)
Free fatty acids (FFA) (%)	1.45±0.05	≤ 1.50
Peroxide value (PV) (mEq/kg)	5.97±0.33	≤ 5.00
p-Anisidine value (AnV) (mEq/kg)	1.01±1.18	≤ 20.00
Total Oxidation (Totoks) (mEq/kg)	12.94±0.84	≤ 26.00

Table 2 Fatty Acid Profile of Raw Tuna Oil

Fatty Acid (% w/w)	Percentage (%)
Butyric Acid (C4:0)	0.09
Myristic Acid (C14:0)	5.39
Pentadecanoic Acid (C15:0)	0.36
Palmitic Acid (C16:0)	13.81
Heptadecanoic Acid (C17:0)	0.38
Stearic Acid (C18:0)	2.46
Arachidic Acid (C20:0)	0.13
Behenic Acid (C22:0)	0.07
Total *Saturated Fatty Acid* (SFA)	22.69
Myristoleic Acid (C14:1)	0.03
Palmitoleic Acid (C16:1)	3.82
Cis-10-Heptadecenoic Acid (C17:1)	0.18
Oleic Acid (C18:1n9c)	11.84
Erucic Acid Methyl Ester (C22:1n9)	10.53
Nervonic Acid (C24:1)	0.80
cis-11-Eicosenoic Acid (C20:1)	0.53
Elaidic Acid (c18:1n9t)	0.40
Total *Monounsaturated Fatty Acid* (MUFA)	28.13
γ-Linolenic Acid (C18:3n6)	0.05
Linolenic Acid (C18:3n3)	0.66
cis-11,14-Eicosadienoic Acid (C20:2)	0.22
Cis-11,14,17-Eicosatrienoic acid ethyl methyl ester (C20:2n-3)	0.71
Arachidonic acid (C20:4n6)	0.58
Cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)	10.09
Cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n-3)	8.05
Total *Polyunsaturated Fatty Acids* (PUFA)	21.58
Total Identified Fatty Acids	72.40

The difference in the appearance of crude tuna oil before and after purification is shown in Figure 1.

Unrefined tuna fish oil has a dark brown color because it still contains impurities. In contrast, refined tuna fish oil is light brown. Magnesol XL, which is used in the bleaching process, can adsorb various impurities in the fish oil, resulting in a lighter color. Magnesol XL is capable of absorbing impurities such as pigments, gums, resins, and peroxide compounds. (Estiasih 2009).

Transesterification of tuna oil

This process aims to improve the stability of fish oil against oxidation. A catalyst is used to accelerate the reaction rate and allow the transesterification process to occur at a relatively low temperature. After obtaining pure fish oil from the transesterification process, the oxidation parameters were analyzed again. As shown in Table 3, the FFA value of the tuna fish oil decreased. This is believed to be because FFA tends to bind with catalyst ions during the transesterification process (Haryani *et al.* 2023). The PV value of pure fish oil has also decreased, likely due to the bleaching process, which can remove undesirable components such as carotenoids and tocopherols (Suseno *et al.* 2021).

Iodine value of omega-3 concentrate and urea crystallization

The use of Design Expert version 13 (DX13) software generated 13 treatment combinations, including 5 center points. The test results are shown in Table 3. A total of 13 treatments were analyzed for quality based on iodine value and yield using the RSM approach. The results showed that all treatments achieved iodine values that met the IFOS (2014) standards. The iodine value indicates the degree of unsaturation of fatty acids in the sample. This is important to know for evaluating the accuracy of the omega-3 concentrate production process. The model specification for the iodine value response is shown in Table 4. The results of the analysis using the RSM approach indicate that the quadratic model is the best model for the iodine value parameter because the sequential p-value is significantly influential ($p < 0.1$) and there is no model misfit indicated by the lack of fit p-value ($p > 0.05$), so it is considered to fit the research data.

Yield value of urea-crystallized omega-3 concentrate

The percentage of omega-3 concentrate obtained is expressed through the yield value parameter. This value serves as an indicator for evaluating the accuracy of the omega-3 concentrate production process. Based on the results of the analysis using the RSM approach, the quadratic model is the best model for the yield parameter. The model specifications for the yield response are shown in Table 5. The sequential p-values are statistically significant ($p < 0.05$). However, the lack-of-fit p-value ($p > 0.05$) indicates a lack of model fit. This means that the resulting model still has limitations in its predictive ability and fit for the yield

Crude oil Pure triglyceride oil

Figure 1 Appearance of crude oil and pure oil.

Table 3 Test results for urea-crystallized omega-3 concentrate

Run	Factor 1 A: Urea ratio (gram)	Factor 2 B: Time (Minutes)	Respon 1 Iodine number (mg/100g)	Respon 2 Yield (%)
1	1.4:1	19 hours 18 minutes	145.62	14.16
2	2.6:1	19 hours 18 minutes	163.15	16.12
3	1.4:1	31 hours 18 minutes	198.28	18.24
4	2.6:1	31 hours 18 minutes	152.93	17.16
5	1.15:1	25 hours 18 minutes	128.40	15.68
6	2.85:1	25 hours 18 minutes	153.66	13.48
7	2:1	16 hours 49 minutes	164.43	7.16
8	2:1	33 hours 47 minutes	216.79	18.08
9	2:1	25 hours 18 minutes	136.99	8.44
10	2:1	25 hours 18 minutes	186.22	9.64
11	2:1	25 hours 18 minutes	195.83	8.68
12	2:1	25 hours 18 minutes	205.54	9.24
13	2:1	25 hours 18 minutes	199.36	8.84

Table 4 Parameter values for the Iodine Number model

Model	*Sequential p-value*	*Lack of Fit p-value*	*Adjusted* R^2	*Predicted* R^2	Description
Linier	0.3925	0.4695	0.0047	-0.4418	
2FI	0.2981	0.4719	0.0261	-0.4136	
Quadratic	0.0914	0.8409	0.3680	0.0729	Recomended
Cubic	0.6259	0.9654	0.2664	0.5122	

response. In general, the results of the RSM approach can still be used to describe the relationship between process variables and the response.

The fatty acid profile of the tuna omega-3 concentrate was reanalyzed, and the results are presented in Table 5. Consistent with the analysis of the crude oil fatty acid profile of tuna, the results of the tuna omega-3 concentrate analysis still showed a predominance of PUFAs, specifically eicosapentaenoic acid (EPA; C20:5n-3). Urea crystallization caused a decrease in total fatty acid content. It is suspected that this is because some PUFAs were trapped in the urea crystal complex during the fractionation process. In addition, the separation and washing processes also caused the loss of the liquid fraction containing some unsaturated fatty acids. The ratio of urea to fatty acids, as well as the temperature and duration of the crystallization process, were found to influence the levels of the fatty acids studied (Von Schacky 2021).

CONCLUSION

After undergoing the purification process, the omega-3 content of the crude tuna oil increased from 19.51% to 32.90%. The response surface methodology (RSM) approach, using a urea ratio of 2:1 and a storage time of 33 hours and 47 minutes, yielded optimal conditions, resulting in the best values for the omega-3 concentrate from tuna. The applied urea crystallization method proved effective in reducing saturated fatty acid content and contributed to an increase in the omega-3 fraction, as indicated by the increased iodine value.

Table 5 Omega-3 Fatty Acid Profile of Urea Crystallization

Fatty Acids (% w/w)	Percentage (%)
Butyric Acid (C4:0)	0.13
Lauric Acid (C12:0)	0.10
Myristic Acid (C14:0)	3.33
Pentadecanoic Acid (C15:0)	0.15
Palmitic Acid (C16:0)	3.23
Stearic Acid (C18:0)	0.53
Total Saturated Fatty Acid (SFA)	7.47
Palmitoleic Acid (C16:1)	5.66
Elaidic Acid (C18:1n9t)	0.65
Oleic Acid (C18:1n9c)	1.81
cis-11-Eicosenoic Acid (C20:1)	0.16
Total Monounsaturated Fatty Acid (MUFA)	8.28
Linoleic Acid (C18:2n6c)	0.98
γ-Linolenic Acid (C18:3n6)	0.16
Linolenic Acid (C18:3n3)	0.21
Cis-8,11,14-Eicosatrienoic Acid (C20:3n-6)	0.15
Arachidonic acid (C20:4n6)	1.15
Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n-3)	12.76
Cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n-3)	4.00
Total Polyunsaturated Fatty Acids (PUFA)	19.41
Total Identified Fatty Acids	35.16

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