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OIL EXTRACTION FROM RICE BRAN USING ETHANOL AND ISOPROPANOL

S M Anisuzzaman*[1], Nurin Nadia Binti Hariffin[2]
[1]Energy and Materials Research Group, Universiti Malaysia Sabah, UMS Road, 88400, Kota Kinabalu, Sabah, Malaysia.
[2]Chemical Engineering Program, Faculty of Engineering, Universiti Malaysia Sabah, UMS Road, 88400, Kota Kinabalu, Sabah, Malaysia.

*** Correspondence:**
anis_zaman@ums.edu.my

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ABSTRACT. *The objective of this study was to extract oil from rice bran using ethanol and isopropanol under different conditions in order to assess and compare their effectiveness. The rice bran oil (RBO) yields were examined at temperatures of 50°C, 60°C, and 70°C, using bran-to-solvent ratios of 1:3, 1:5, and 1:7 and extraction durations of 2.5, 5, and 7.5 hrs. The Soxhlet extraction method was employed for the extraction, followed by solvent separation using a rotary evaporator. The isopropanol at 70°C, with a ratio of 1:7 and a reaction time of 5 hrs, achieved the highest yield of 14.87% using this method. Under the same conditions, ethanol had a yield of 12.04%. The physical and chemical characteristics of the extracted oil were thoroughly examined using Fourier transform infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GCMS) analysis, acid value, free fatty acid, and peroxide value. The analysis showed that the acid values for ethanol and isopropanol were* 27.53 *mg KOH/g and* 23.90 *mg KOH/g, respectively. The percentages of free fatty acids were* 13.77% *(ethanol) and* 11.95% *(isopropanol). The peroxide value assays, which indicate the oxidative stability, exhibited notable variation: the isopropanol-extracted RBO had lower peroxide values ranging from* 111.20 *to* 192.40 *meq/kg, whereas the ethanol-extracted RBO had peroxide values ranging from* 194.40 *to* 222.40 *meq/kg. The data were analyzed via response surface methodology (RSM) to optimize the extraction parameters, forecasting the maximum yield at a temperature of 70°C, a ratio of bran to oil of 1:7, and an extraction duration of 5 hrs. Because the p-value (0.2706) was higher than 0.05, the ANOVA findings indicated that the regression model was not statistically significant.*

INTRODUCTION

Rice bran, the outermost pericarp of the rice kernel, is a byproduct of rice milling that is abundant in nutrients and holds considerable promise for diverse industrial uses. Of all these options, the process of obtaining rice bran oil (RBO) is especially intriguing because of its well-known health advantages and its wide range of applications in the culinary, medicinal, and cosmetic sectors.

Various methods exist for oil extraction, each possessing distinct benefits and constraints. Soxhlet extraction is a conventional technique that employs a solvent to iteratively cleanse the solid substance in order to extract the targeted chemical. This method utilizes a Soxhlet device, which enables uninterrupted extraction of the solvent, guaranteeing complete extraction of the desired component from the solid material **(**Shi *et al.*, 2023). Supercritical fluid extraction (SFE) utilizes supercritical fluids, such as CO_2, as solvents for extracting chemicals. This method offers selectivity, speed, and the capability to extract thermally labile molecules without causing deterioration (Baena-Moreno *et al.*, 2018). Mechanical pressing, specifically cold pressing, entails the physical compression of the source material to extract oils without the application of heat or chemicals. This method preserves the nutritional and sensory attributes of the oil; however, it may result in a lower oil yield **(**Choo *et al.*, 2015**).** Enzyme-assisted aqueous extraction uses enzymes to degrade cell walls and liberate oil into a water-based phase, thereby enhancing both the quantity and quality of the yield in an eco-friendly manner (Yusoff *et al.*, 2015**).** Ultrasound assisted extraction (UAE) employs ultrasonic waves to augment the infiltration and disruption of the plant material, hence enhancing extraction efficiency (Chemat *et al.*, 2018)**.** Microwave assisted extraction (MAE) uses microwave radiation to heat the solvent and plant material, thereby improving extraction efficiency, reducing solvent usage, and offering a rapid and efficient method for extracting thermally sensitive chemicals (Kumar *et al.*, 2018)**.**

Hexane is traditionally the most used solvent for extracting RBO. However, there are substantial safety and environmental issues due to the method's frequent usage of hexane. Hexane is a volatile organic compound (VOC), which raises environmental issues due to its propensity to contaminate the air and impair human health (Kumar *et al*., 2017). Additionally volatile and explosive, hexane presents hazards in manufacturing facilities. Finding new environmentally friendly, safe, and sustainable methods to extract RBO is necessary because of the detrimental effects hexane has on the environment and human health. The use of ethanol and isopropanol as solvents is one of the significant options being studied. Alcohols like ethanol and isopropanol have drawn interest as prospective alternatives to hexane because of their non-toxic, biodegradable, and renewable properties (Capellini *et al.*, 2017). Also, these alcohols are consistent with the global push towards environmentally friendly and sustainable extraction methods. Ethanol can efficiently dissolve the lipids in rice bran, which suggests that it could be a more environmentally friendly substitute for hexane (Capellini *et al.*, 2017). Isopropanol contains alcohol and has shown promise as a safe and efficient alternative for extracting oil (Subra-Paternault *et al.*, 2022). Despite the promising features of ethanol and isopropanol for RBO extraction, there is a noticeable dearth of literature on their application. However, these properties are in line with the global trend towards sustainable and ecologically friendly extraction technologies. The practicality, effectiveness, and environmental effects of using these solvents for extracting RBO remain unknown, despite previous studies on their use on other oil sources (Fraterrigo *et al*., 2021).

In order to increase the amount, effectiveness, and quality of the extracted oil, optimization studies are essential in RBO extraction. The utilization of response surface methodology (RSM) is necessary in order to systematically and statistically assess the influence of various elements and their interactions (Majid *et al*., 2020, Veza *et al*., 2023). RSM aids in determining the most favorable parameters for the extraction process, guaranteeing the highest possible output and desired level of quality while minimizing resource and time consumption (Veza *et al*., 2023).

Hence, the objective of this study was to evaluate the yield of RBO by employing solvents, such as ethanol and isopropanol, under different conditions. Additionally, the oil was characterised through Fourier transform infrared spectroscopy (FTIR) and gas chromatography mass spectrometry (GCMS) analysis, while the peroxide value (PV) was measured to determine the extent of oxidation. Furthermore, the extraction parameters, including temperature, time, and rice bran-to-solvent ratio, were optimised using RSM.

MATERIALS AND METHODS

Raw Materials Collection and Preparation

The rice bran was acquired from one of the local rice mills in Sabah, Malaysia. To get rid of any undesired foreign materials such as broken grains, hull pieces, and paddy kernels, the bran was put through a sieve. Then, to avoid enzymatic rancidity, the rice bran was stabilized after sifting. The rice bran was dried by heating it to 115°C for 10 min in a dry oven. The primary purpose of the drying oven was to stabilize rice bran by deactivating the lipase enzyme, which is responsible for causing rancidity in the bran. The rice bran stabilization condition is consistent with reported thermal stabilization approaches for rice bran, where short-duration dry heat treatment is commonly applied to inactivate lipase and prevent rapid free fatty acid formation. Literature reports that rice bran stabilization is typically achieved using dry heat conditions within the range of 110–120°C, depending on processing method and moisture content (Lv *et al.*, 2017).

Apparatus and Chemicals

The Soxhlet extractor is a device used specifically for the extraction of oil. The Rotavapor type R-215 was designed for the purpose of separating solvents and oils. The solvent employed was ethanol, identified by its CAS number 64-17-5, which possesses a high purity level of 99.8%. Isopropanol, also known as CAS No. 67-63-0, has a purity level of 99.9%. Classic Chemicals Sdn Bhd, Malaysia, provided both solvents. The GCMS model Shimadzu was used for the chemical analysis of the RBO. On the other hand, FTIR (Perkin Elmer) was used to identify functional groups present in the extracted oil samples.

RBO Extraction Utilising Ethanol and Isopropanol

The Soxhlet equipment was used to extract the oil from rice bran. A ratio of rice bran to solvent of 1:3 was added to the round-bottomed flask, and a sample weighing 50 g of rice bran was put into the thimble and covered with gauze on top. For 2.5, 5, and 7.5 hrs, the extraction procedure was carried out at different temperatures, 50, 60, and 70°C, and at different ratios of bran to solvent, 1:3, 1:5, and 1:7 w/v. After filtering the extract with filter paper, it was separated from the solvent using a rotary evaporator (Pimpa *et al.*, 2021). The selected time and temperature parameters were derived based on the boiling points of ethanol and isopropanol, as well as the recommendations provided by RSM. The Soxhlet extraction was conducted under continuous reflux for varying durations, followed by rotary evaporation is consistent with commonly applied laboratory-scale oil extraction conditions. Although the exact number of cycles was not explicitly monitored, typical Soxhlet cycle times range between 10–20 mins depending on solvent and heating conditions; thus, the process is estimated to involve approximately 7–45 cycles across the experimental time range. Residual solvent content was not quantitatively determined, however, rotary evaporation at controlled temperature was widely recognized as effective for substantial solvent removal in lipid extraction systems. In order to minimize the amount of residual solvent in the extracted RBO, various steps and factors were taken into account. Ethanol and isopropanol were selected due to their distinct boiling points, which enable effective separation from the oil. The extraction parameters, such as duration and temperature, were determined by considering the boiling points of the substances involved and following the suggestions provided by RSM. Following the recommended temperature ranges provided by RSM enhances the extraction process to attain optimal efficiency while guaranteeing successful removal of the solvent (Aydar, 2018). Next, the filtered extract was processed using a rotary evaporator, which decreases the pressure and decreases the boiling point of the solvent. This enables the solvent to evaporate at a lower temperature, leaving behind only pure oil (Bennour *et al.*, 2020; Kolesnichenko *et al.*, 2019). Each experimental condition was performed as a single experimental run due to material and resource constraints.

Determination of Oil Yield

Following the extractions, the samples were subjected to double filtration using a Whatman paper filter of grade one. This process aimed to effectively separate the oil from the spent rice bran. Subsequently, the solvent was evaporated. The RBO yield was computed using equation 1 (Javed *et al.*, 2014):

$$RBO\ yield, \%(w/w) = \frac{WRBO(g)}{WRB(g)} \times 100 \tag{1}$$

where WRBO (g) represents the weight of RBO obtained from the experiment, and WRB (g) represents the weight of the rice bran prior to the extraction of oil.

Characterization of Oil Extracted and Analysis

Determination of PV of RBO

First, a 250 mL Erlenmeyer flask weighed down to contain 2.5 g of each sample was sealed right away with a glass stopper. A 15 mL mixture of chloroform solution and acetic acid was added in a 3:2 ratio. Before adding the solution to 0.5 mL of potassium iodide (KI), the mixture was agitated. To make the KI solution, the KI solute was added drop by drop to distilled water until it became saturated. The solution was then allowed to react for 60 seconds ± 1 second while being vigorously stirred by hand at least twice. The solution was then combined with 15 mL of distilled water and 0.5 mL of starch indicator. 2 g of starch was combined with 100 mL of hot distilled water to create a starch indicator. After adding the saturated KI mixture to the sample, the amount of iodine that was released from KI due to the oxidative action of oil-soluble peroxides was measured by titrating standard 0.1 N sodium thiosulphate while utilizing the starch solution as an indicator. Additionally, titration was done for the blanks. With 0.1 N sodium thiosulphate, the solution was titrated until it changed from an oily yellow to a colourless state. Three duplicates of each sample were prepared (IFRA, 2019). PV was calculated using equation 2:

$$PV, \text{meq/kg} = \frac{(V_1 - V_0) \times c \times 1000 \times T}{m} \tag{2}$$

where V_1 represents the consumption of sodium thiosulfate solution in the main test in mL, and V_0 represents the consumption of sodium thiosulfate solution in the blank test in mL. c represents the molar concentration (molarity) of the sodium thiosulfate solution in mol/L. T represents the titer of the thiosulfate solution while m is weighed portion of substance in g.

Determination of Free Fatty Acid Value (AV)

Both oil samples AV and percentages of free fatty acids (FFA, %) were calculated using the methods outlined by Asmare & Gabbiye (2014). After calculating the AV, equation 3 was used to determine the FFA content in the RBO.

$$FFA, \% = AV/2 \tag{3}$$

To find the AV, 25 mL of a 1:1 mixture of diethyl ether and ethanol was added to a 250 mL conical flask containing 5 g of oil. The mixture was then mixed well. There are five drops of phenolphthalein indicator added to the solution, and 0.1 N KOH was used to test its strength. The endpoint of the titration was proved when the solution kept shaking and turned pink from colourless. During the titration, the amount of 0.1 N KOH (V) used was written down. The method was used to find the sample's total acidity, which is given as mg KOH/g (Asmare & Gabbiye, 2014).

$$AV = \frac{56.1 \times N \times V}{WRBO} \tag{4}$$

The variable N represents the normality of the KOH solution, V represents the volume of ethanolic KOH in mL, and $WRBO$ represents the weight of the RBO sample in g.

GCMS Analysis

The goal of doing GCMS analysis on the RBO is to identify and quantify the various types of fatty acids contained in the RBO. The sample was prepared for GCMS analysis by utilising methanol of HPLC grade as the solvent. Methanol was chosen for its exceptional purity and ability to work well with various analytes. Several stock preparations were employed to provide accurate and reliable results throughout the preparation of the RBO sample for GCMS analysis. The preparation process commenced by creating three separate stock solutions, namely stock A, stock B, and stock C.

Three separate stock solutions, stock A, stock B, and stock C, were constructed as the first step in the preparation process. 1 mL of the RBO sample and 9 mL of methanol were combined to create stock A. This dilution step was required to produce the RBO sample at the proper concentration for the assay. To create stock B, 0.5 mL of stock A was then further diluted with 9.5 mL of the solvent. This additional dilution allowed for the proper concentration range required for the GCMS investigation. To make stock C, 0.2 mL of stock B was diluted with 9.6 mL of the solvent. The stock C sample was then ready for GCMS analysis by being placed into a vial. For the analysis, the sample's vial is meticulously placed into the gas chromatograph equipped with an MS detector. A gas chromatograph equipped with an MS detector was utilized to perform the GCMS analysis. The GCMS is configured with a rate temperature of 140°C and a hold duration of 10 min. The equilibrium time for the analysis was set at 20 mins. The temperature was subsequently raised from that point to 250°C, increasing at a rate of 70°C per minute, and then held at that temperature for 10 min. The analysis was conducted under specific conditions, utilizing a splitless injection mode with injection temperatures set at 250°C and a split pressure of 100 kPa, with a split ratio of 75:1. The temperature of the mass spectrometer (MS) was set at 220°C, while the temperature of the interface was maintained at 280°C. A solvent delay or cut time of 3.40 min was implemented to guarantee accurate separation and detection of the chemicals. The mass spectrometer was set to collect data within the mass-to-charge ratio (m/z) range of 50 to 500.

FTIR Analysis

Using FTIR for the extraction of oil from rice bran using ethanol and isopropanol can provide several expected results. FTIR spectroscopy can identify functional groups present in the extracted oil samples. Besides, FTIR can be used to quantify the oil content in the extracted samples by analyzing specific absorption peaks associated with oil molecules. Moreover, FTIR can detect the presence of residual ethanol and isopropanol solvents in the extracted oil samples (Irnawati *et al.*, 2020).

Optimization Using RSM

The ideal Soxhlet extraction parameters were determined using a three-factor, three-level Box-Behnken design (BBD), taking into account the extraction temperature, time, and rice bran to solvent ratio. BBD allows for a decreased number of experiments, which leads to a more efficient and cost-effective approach (Granato & Veronica, 2014). Table 1 presents the extraction variables together with their corresponding levels and coded factors.

Table 1. Coded values of process parameters and corresponding responses

Symbol	Parameter	Units	Level -1	Level 0	Level 1
$X1$	Extraction temperature	ºC	50	60	70
$X2$	Bran to solvent ratio	-	1:3	1:5	1:7
$X3$	Extraction time	h	2.5	5	7.5
Y	RBO yield	%	Y1	Y2	Y3

RESULTS AND DISCUSSION

Effect of Different Extraction Temperatures on RBO Yield

In order to examine the impact of extraction temperature on RBO extraction, Figure 1 displays the experimental results for the extraction condition under different temperatures for both solvents at 50, 60, and 70°C. The other two conditions of extraction time and rice bran to solvent ratio were kept constant at 5 hrs and a 1:5 bran to solvent ratio.

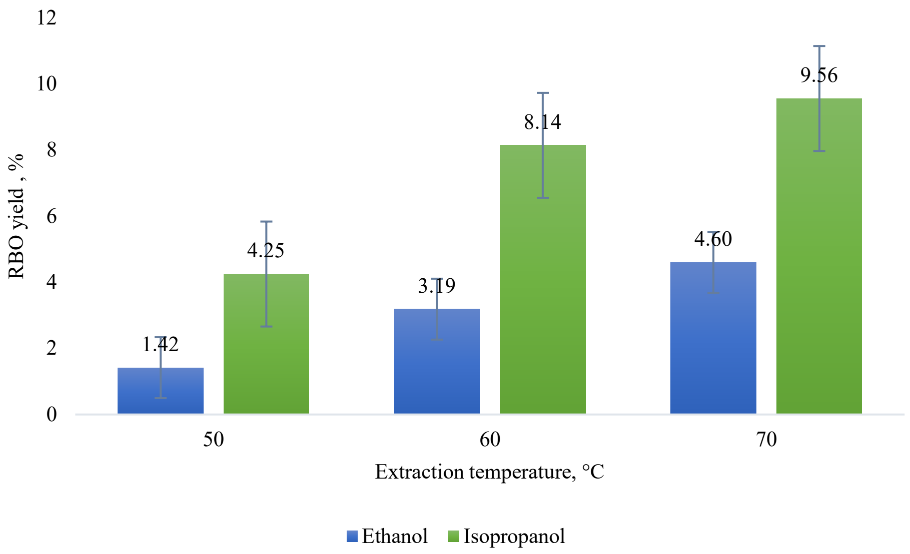


Figure 1. Effect of extraction temperature on RBO yield

Based on Figure 1, both ethanol and isopropanol showed an increase in yield of RBO when the extraction temperature increased. At 50°C, ethanol produced 1.42% RBO, but isopropanol produced 4.25% RBO. When the extraction temperature was raised to 70°C, the RBO yields increased significantly to 4.60% and 9.56% for ethanol and isopropanol, respectively. According to Djaeni & Listyadevi, (2019), the highest yield ultrasound-assisted extraction was 20.35% with an ideal bran to solvent ratio of 1:5 w/v using 60°C. Due to its polarity and relatively low boiling point (78.37°C), ethanol is highly efficient at extracting polar molecules. On the other hand, isopropanol, which is likewise a polar solvent but has a higher boiling point (82.6°C), may be heated to higher temperatures without experiencing substantial evaporation. Isopropanol often produces a greater amount of RBO than ethanol when exposed to higher temperatures because it has better solvent characteristics and a higher boiling point (Junyusen *et al.*, 2022).

Effect of Different Rice Bran and Solvent Ratios on RBO Yield

The experiment results for the extraction conditions under different rice bran to solvent oil ratios for both solvents at 1:3, 1:5, and 1:7 are displayed in Figure 2. The other two conditions of extraction time and extraction temperature were kept constant at 5 h and 70°C to examine the impact of the different rice bran to solvent ratios on the RBO extraction.

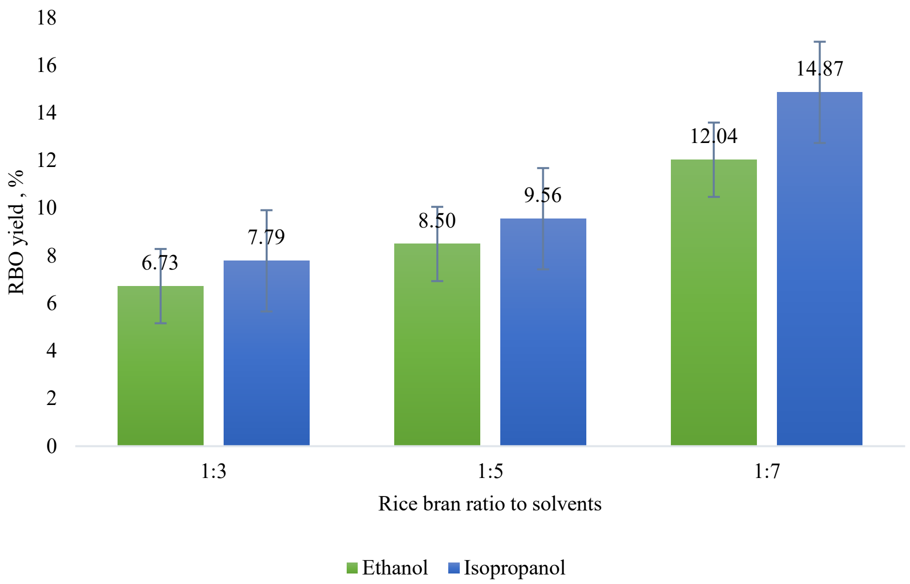


Figure 2. Effect of rice bran ratio to RBO yield

Based on Figure 2, the RBO yield increases as the solid-liquid ratio increases for both ethanol and isopropanol solvents. Isopropanol yielded 7.79% at a ratio of 1:3, ascended to 9.56% at a ratio of 1:5, and increased further to 14.87% at a ratio of 1:7. Similarly, the RBO yield for ethanol increased from 6.73% at a ratio of 1:3 to 8.50% at a ratio of 1:5 and 12.04% at a ratio of 1:7. This condition is caused by an oil concentration difference between the surface of the rice bran and the solvent. According to Djaeni & Listyadevi (2019), when the ratio of rice bran to solvent was 1:2 (w/v), the yield was 15%. In addition, when the ratio was increased to 1:5 (w/v), the yield also climbed to 20%. However, increasing the ratio beyond 1:5 (w/v) led to a loss in yield. As the ratio of solid to liquid increases, the effectiveness of the solvent also increases because there are a larger contact area and a more pronounced concentration gradient (Savic Gajic *et al.*, 2021).

Effect of Different Extraction Times on RBO Yield

In order to examine the impact of the different rice bran to solvent ratios on the RBO, Figure 3 displays the experimental results for the extraction condition under different extraction times for both solvents at 2.5, 5, and 7.5 hrs. The other two conditions, rice bran to solvent ratio and extraction temperature, were kept constant at 1:5 and 50°C.

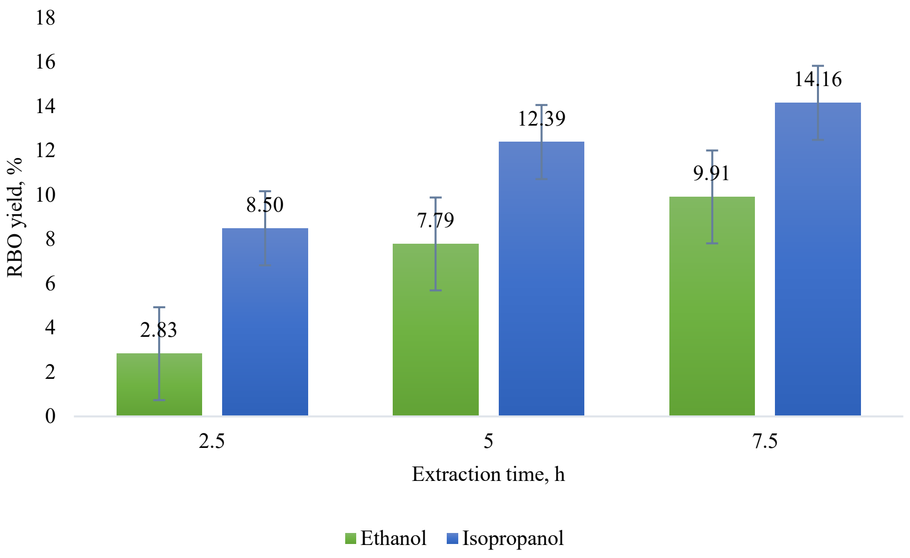


Figure 3. Effect of extraction time (h) to RBO yield

From Figure 3, it can be observed that the RBO yield increased with longer extraction times for both ethanol and isopropanol solvents. For isopropanol, the RBO yield was 8.50% at an extraction time of 2.5 h, which increased to 12.39% at 5 h and further increased to 14.16% at 7.5 h. Similarly, for ethanol, the RBO yield increased from 2.83% at 2.5 h to 7.79% at 5 h and reached 9.91% at 7.5 h. Elkhaleefa & Shigidi (2015) provide an explanation for these results, demonstrating that increasing the duration of contact between the sample and the solvent leads to a higher percentage of extraction yield. Isopropanol, with its enhanced boiling point and superior solvent characteristics at higher temperatures, consistently generates greater yields compared to ethanol, especially during longer extraction periods (Shukla *et al*., 2017).

In nutshell, isopropanol at 70°C, with a ratio of 1:7 and a reaction duration of 5 h, achieved the highest yield of 14.87%. Under the same conditions, ethanol had a yield of 12.04%. The labeling of these two RBO samples was made as RBO-I-7 and RBO-E-7, respectively and these samples were used for characterisation of extracted RBO.

Characterization of Extracted RBO

Physiochemical Analysis

Table 2 displays the results of the AV and FFA% analysis conducted on both samples of RBO.

Table 2. Physical analysis result of RBO sample

Properties	Ethanol	Iso-propanol
AV, mg KOH/g	27.53	23.90
FFA, %	13.77	11.95

The oil extracted using ethanol exhibits slightly higher acid value (AV) and free fatty acid (FFA%) compared to the oil extracted using isopropanol. The AV of ethanol is 27.53 mg KOH/g, while isopropanol shows a lower value of 23.90 mg KOH/g, indicating a comparatively lower acidity level in the isopropanol-extracted oil. Similarly, the FFA% values for ethanol and isopropanol are 13.77% and 11.95%, respectively. This demonstrates that ethanol extraction results in a higher accumulation of FFA than isopropanol under the same experimental conditions. Both samples shown elevated AV and FFA% values compared to typical literature reports for crude RBO, where FFA is generally around 1–5%, suggesting partial oil degradation or the presence of more reactive lipid fractions in the extracted samples (Orthoefer, 2020). Table 3 illustrates the PVs of RBO samples extracted using isopropanol and ethanol under different conditions.

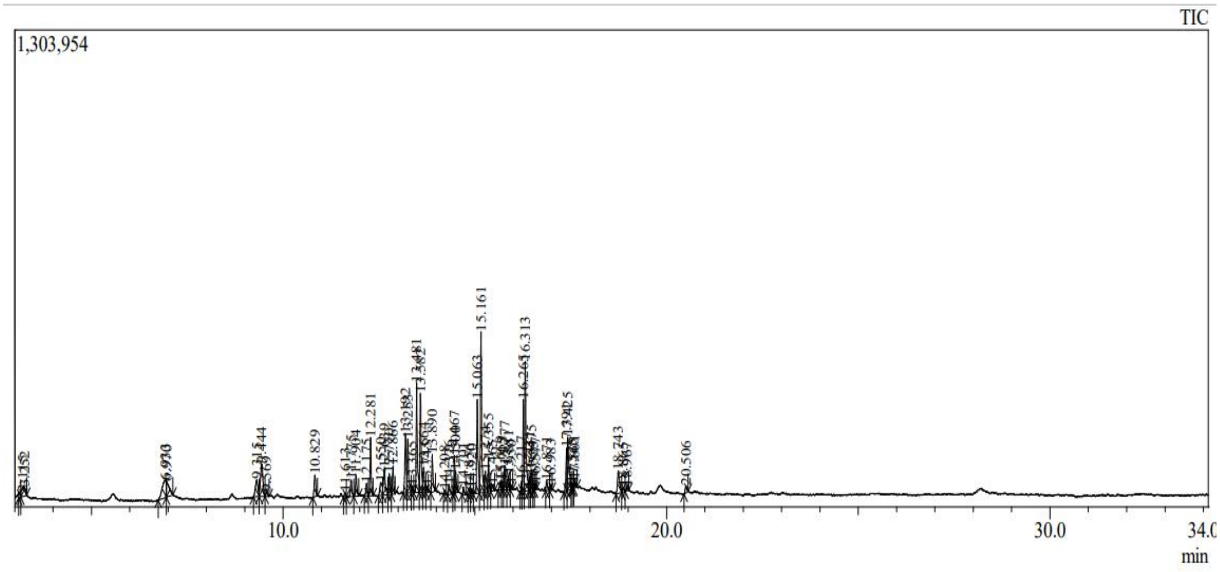
Table 3. PV result of RBO samples

	Testing for temperature (70°C)		**Testing for the ratio of bran to solvent (1:7)**		**Testing for time (5 h)**	
	Isopropanol	**Ethanol**	**Isopropanol**	**Ethanol**	**Isopropanol**	**Ethanol**
PV, meq/kg	111.20	199.20	174.00	222.40	192.40	194.40

As shows Table 3, isopropanol extraction consistently yielded lower PV compared to ethanol extraction. At a constant temperature of 70°C, isopropanol demonstrated lower PV, suggesting that it may be less conducive to oxidation at this temperature compared to ethanol. Likewise, at the same ratio of bran to solvent (1:7), isopropanol extraction generally resulted in reduced PV, indicating potentially lower extraction efficiency or solubility of peroxide compounds compared to ethanol. However, intriguingly, at a testing time of 5 hrs, isopropanol extraction exhibited slightly higher PV. Elevated PV indicates accelerated oxidation, leading to oil breakdown during transesterification. Additionally, highly oxidized oil yields biodiesel with reduced stability, prone to further oxidation and increased viscosity (Kansedo *et al.*, 2009].

GCMS Analysis

The results of the study of the concentration of fatty acids in RBO may be seen in Figure 4, which was obtained from sample RBO-I-7.

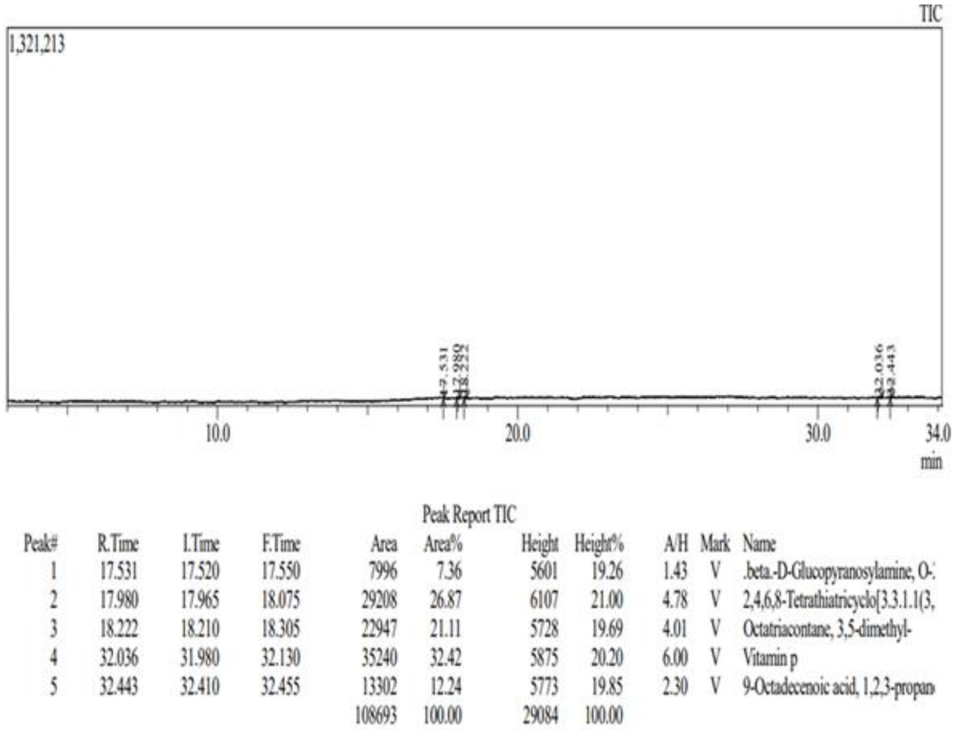


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.115	3.100	3.160	55330	0.50	14997	0.34	3.69	V	Butanoic acid, 4-chloro-, 1,1a,1b,4
2	3.252	3.160	3.315	165066	1.48	22910	0.51	7.20	V	2-(2',4',4',6',6',8',8'-Heptamethyltet
3	6.933	6.740	6.955	353247	3.17	46429	1.04	7.61		Bis(pentamethylcyclotrisiloxy)tetra
4	6.970	6.955	7.125	212877	1.91	41126	0.92	5.18	V	Bis(pentamethylcyclotrisiloxy)tetra
5	9.315	9.225	9.375	210725	1.89	46121	1.04	4.57		Heptasiloxane, hexadecamethyl-

Figure 4. GCMS analysis result of sample RBO-I-7

On the chromatogram there is a graph containing a peak that shows the composition and the amount of the constituent samples of RBO. On the graph, the 'X' axis indicates retention time or component separation time, while the 'Y' axis shows the abundance of certain substances contained in the injected sample. Based on the GCMS analysis of RBO extracted, several peaks were identified, revealing important insights into the composition and potential contamination of the sample. Peak 1, identified as Butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahyd, was typically found in various natural extracts and essential oils, often resulting from microbial activity or degradation processes. Its presence in RBO could be due to microbial contamination or the breakdown of fatty acids during storage and extraction. Peak 2, identified as 2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4-, points to the presence of siloxane compounds, commonly used in industrial applications such as lubricants and cosmetics. This suggests contamination from industrial processing equipment or packaging materials used during the extraction process. Peak 3, Bis(pentamethylcyclotrisiloxy)tetramethyldisiloxane, indicates significant contamination, likely from silicone-based lubricants or antifoaming agents used during extraction. The high abundance of this compound, along with its recurrence in Peak 4, underscores the extent of contamination. Peak 5, heptasiloxane, hexadecamethyl-, further confirms contamination from industrial materials (McNair & Miller, 2009). The presence of multiple siloxanes suggests systemic contamination, possibly originating from the extraction equipment or storage containers. Improper storage and the use of industrial-grade solvents and equipment not specifically designed for food processing can introduce these contaminants into the oil (Raje *et al*., 2019). Figure 5 displays the results of a GCMS analysis for a sample which taken from sample RBO-E-1:7.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	17.531	17.520	17.550	7996	7.36	5601	19.26	1.43	V	.beta.-D-Glucopyranosylamine, O-
2	17.980	17.965	18.075	29208	26.87	6107	21.00	4.78	V	2,4,6,8-Tetrathiatricyclo[3.3.1.1(3,
3	18.222	18.210	18.305	22947	21.11	5728	19.69	4.01	V	Octatriacontane, 3,5-dimethyl-
4	32.036	31.980	32.130	35240	32.42	5875	20.20	6.00	V	Vitamin p
5	32.443	32.410	32.455	13302	12.24	5773	19.85	2.30	V	9-Octadecenoic acid, 1,2,3-propan
				108693	100.00	29084	100.00			

Figure 5. GCMS analysis result of sample RBO-E-1:7

The first peak, observed at a retention time of 17.53 min, represents beta.-D-Glucopyranosylamine, O-. This compound has an area of 7996, which accounts for 7.36% of the total area, and a height of 5601, corresponding to 19.26% of the total height, with an A/H ratio of 1.43. The second peak, appearing at a retention time of 17.98 min, was identified as 2,4,6,8-Tetrathiatricyclo[3.3.1.1(3, with an area of 29208 (26.87%) and a height of 6107 (21.00%), resulting in an A/H ratio of 4.78. The third peak, at 18.222 minutes, was attributed to Octatriacontane, 3,5-dimethyl-, which has an area of 22947 (21.11%) and a height of 5728 (19.69%), with an A/H ratio of 4.01. The fourth peak, observed at 32.036 min, corresponds to Vitamin P. This peak has the highest area of 35240 (32.42%) and a height of 5875 (20.20%), leading to an A/H ratio of 6.00. Lastly, the fifth peak, at 32.443 min, was identified as 9-octadecenoic acid, 1,2,3-propan, with an area of 13302 (12.24%) and a height of 5773 (19.85%), resulting in an A/H ratio of 2.30.

FTIR Analysis

Figures 6 and 7 show FTIR analysis results of samples RBO-I-7 and RBO-E-1:7, respectively.

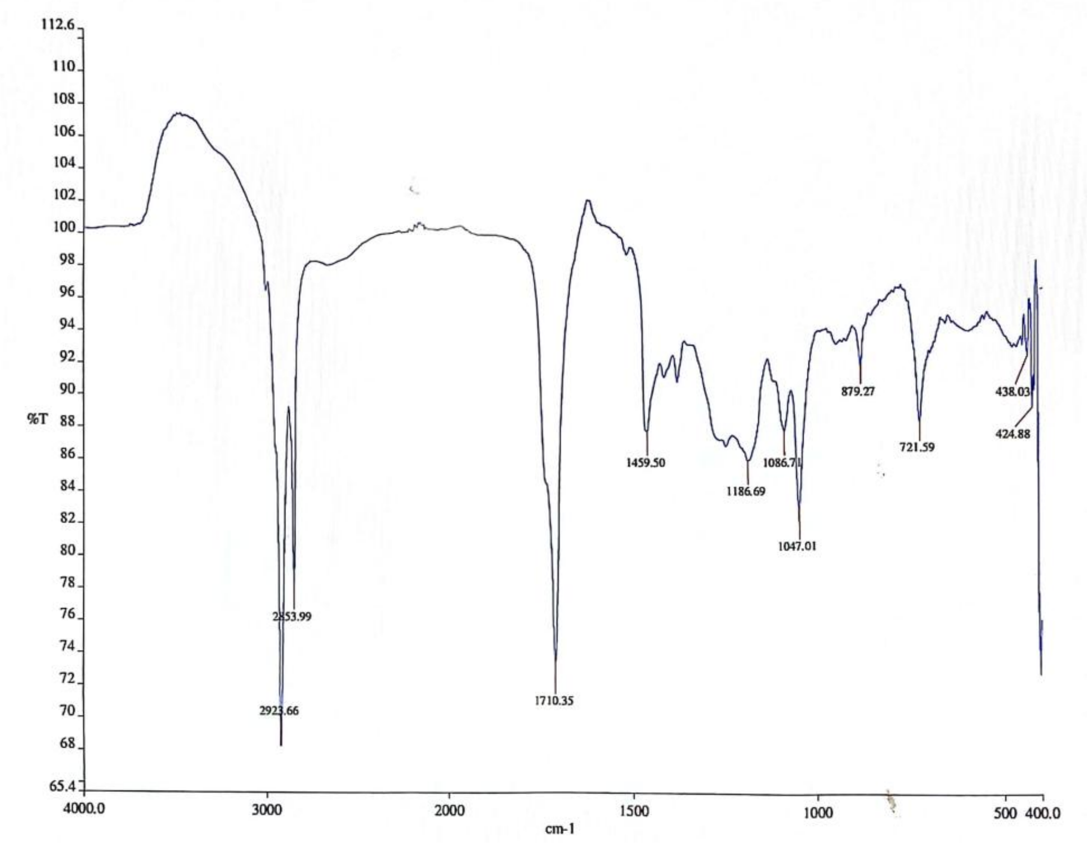


Figure 6. FTIR analysis result of sample RBO-I-7

Multiple prominent peaks were seen in the FTIR spectra of RBO. The peak observed at 2923.66 cm^{-1} corresponds to the asymmetric stretching vibrations of methylene ($-CH_2-$) groups, which are commonly found in long-chain hydrocarbons, including fatty acids and triglycerides. The presence of symmetric stretching vibrations of these methylene groups is responsible for a corresponding peak at 2853.99 cm^{-1}. The prominent absorption peak at 1710.35 cm^{-1} was attributed to the stretching vibrations of carbonyl ($C=O$) groups in ester bonds, indicating the presence of triglycerides. Triglycerides are the main components of vegetable oils. The peak at 1459.50 cm^{-1} was caused by the bending vibrations of methylene ($-CH_2-$) and methyl ($-CH_3$) groups, which are present in the hydrocarbon chains of fatty acids. The existence of ester groups is additionally verified by a peak at 1047.01 cm^{-1}, indicating the existence of unsaturated fatty acids in the RBO. Finally, the peak at 721.59 cm^{-1} corresponds to the oscillations of extended methylene groups, suggesting the existence of long hydrocarbon chains in fatty acids and triglycerides. Conversely, the findings presented by Zuñiga-Díaz *et al.* (2017) offer a slightly different FTIR spectrum for RBO.

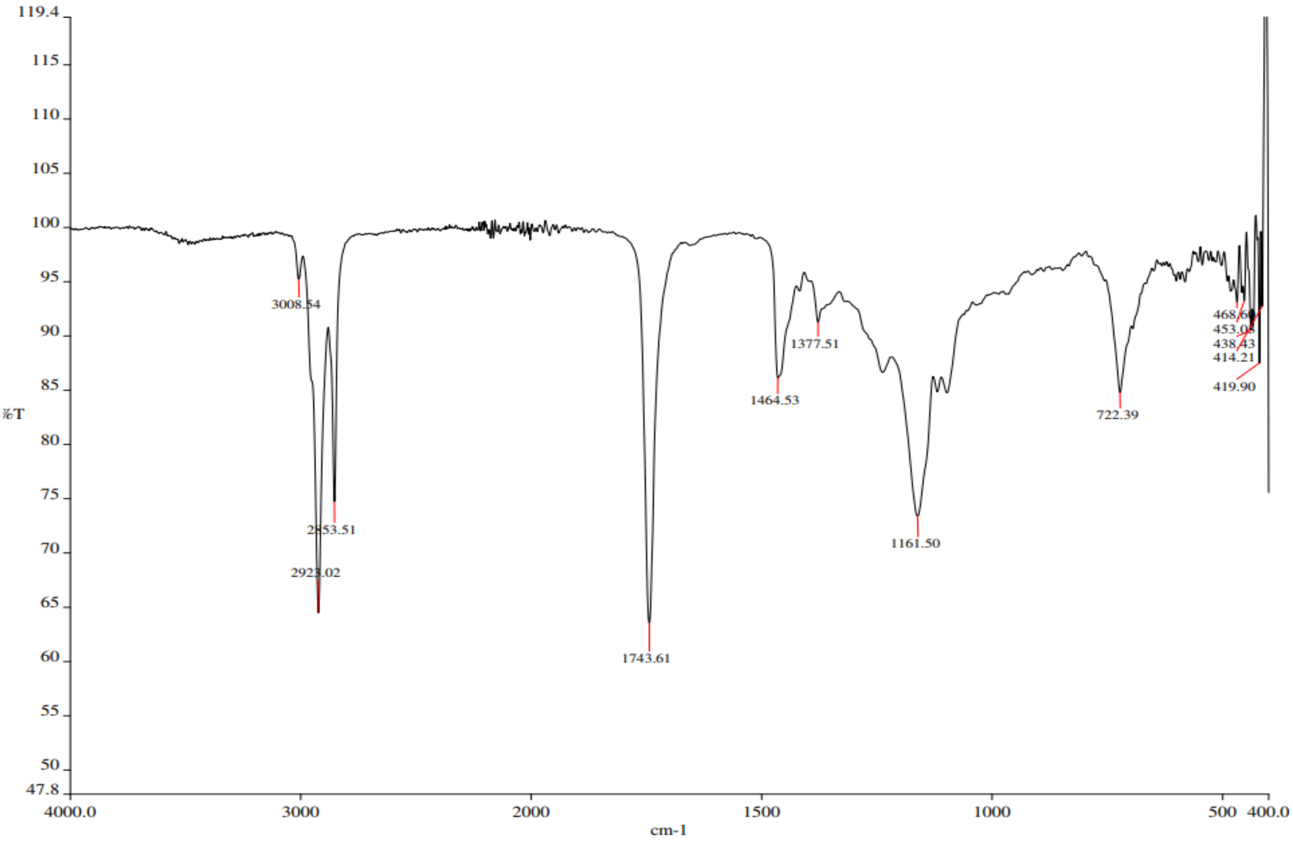


Figure 7. FTIR analysis result of sample RBO-E-1:7

A peak at 3008.54 cm^{-1} indicates the presence of C-H stretching vibrations, which are commonly found in alkenes (C=C-H). This indicates the existence of carbon chains that contain double or triple bonds. The peaks seen at 2922.02 cm^{-1} and 2853.51 cm^{-1} correspond to the stretching vibrations of C-H bonds in aliphatic hydrocarbons. These peaks especially indicate the presence of methylene (CH_2) and methyl (CH_3) groups. The prominent absorption at 1743.61 cm^{-1} corresponds to the intense C=O stretching vibration, which is a distinctive feature of carbonyl groups commonly present in ketones, aldehydes, or esters. The presence of this peak indicates the presence of one or more functional groups in the sample. The presence of a peak at 1161.50 cm^{-1} suggests the occurrence of C-O stretching vibrations, which are typical in alcohols, ethers, esters, and carboxylic acids. This indicates the possible presence of these functional groups in the sample. A peak at 722.39 cm^{-1} suggests the presence of long-chain methylene rocking vibrations, which are commonly observed in long-chain aliphatic compounds. Peaks observed at 468.67 cm^{-1}, 453.09 cm^{-1}, 438.43 cm^{-1}, and 414.21 cm^{-1} in the lower wavenumber area may be associated with different skeletal vibrations.

Both ethanol- and isopropanol-extracted RBO samples exhibit characteristic lipid functional groups, including strong absorption bands near ~1740 cm^{-1} corresponding to C=O stretching of ester groups, confirming the presence of triglycerides. The isopropanol sample shows a peak at 1710.35 cm^{-1}, while the ethanol sample exhibits a slightly shifted peak at 1743.61 cm^{-1}, indicating minor variation in carbonyl environment or oxidation level. In the C–H stretching region (~2920–2850 cm^{-1}), both samples display similar peaks, although the ethanol-extracted sample shows slightly higher intensity, suggesting a relatively higher hydrocarbon content or extraction efficiency. Notably, in the fingerprint region (1000–1200 cm^{-1}), the ethanol sample (1161.50 cm^{-1}) shows stronger absorption compared to the isopropanol sample (~1186.69 and 1086.71 cm^{-1}), which may indicate a higher concentration of ester or alcohol-related functional groups. Overall, while both solvents extract similar functional groups, the ethanol-extracted oil demonstrates slightly higher peak intensities in key regions, supporting earlier findings of higher FFA and acid value, which may indicate increased lipid hydrolysis or compositional variation.

Statistical Analysis and Optimization

The RBO extraction method was subjected to statistical analysis and parametric optimization utilizing the best suitable solvent, which in this study was isopropanol. The RSM was used to optimize and design experiments and the details of this design may be found in Table 4.

Table 4. Design of experiment for three independent variables and experimental results

Run	Extraction temperature, $X1$		Solid-to-solvent ratio, $X2$		Extraction time, $X3$		RBO yield, Y	Predicted RBO yield
	Coded value	Actual value	Coded value	Actual value	Coded value	Actual value		
1	-1	50	0	1:5	0	5	4.25	9.62
2	0	60	0	1:5	0	5	8.14	9.79
3	1	70	0	1:5	0	5	9.56	9.97
4	1	70	-1	1:3	0	5	6.73	5.90
5	1	70	0	1:5	0	5	9.56	9.97
6	1	70	1	1:7	0	5	14.87	14.04
7	-1	50	0	1:5	-1	2.5	8.50	6.79
8	-1	50	0	1:5	0	5	12.39	9.62
9	-1	50	0	1:5	1	7.5	14.16	12.45

The RBO extraction process was proposed to be modelled using a linear model. The resulting linear model, expressed in terms of coded variables, is as follows:

$$Y = 9.794 + 0.177X1 + 4.071X2 + 2.832X3 \quad (5)$$

The model equation's coefficients, namely 0.177, 4.071, and 2.832, represent the individual impact of each process variable on the response. The correlation coefficient of 0.177 for $X1$ suggests that a one unit increase in the coded value of temperature $X1$ leads to a 0.177 unit increase in the percentage yield of RBO, assuming that the other variables $X2$ and $X3$ remain unchanged. The coefficients of $X2$ and $X3$, which are 4.071 and 2.832, respectively, demonstrate the impact of the solvent-bran ratio and extraction duration on the response.

Model Fitting and Summary Statistics

The mathematical model generated using the RSM approach was validated through experiments conducted with the specified optimal medium characteristics. To assess the model's usefulness and accuracy, a statistical t-test was conducted using various statistical indicators, including the coefficient of determination (R^2), Adjusted R^2, and root mean square error (RMSE). Table 5 and 6 present the condensed information regarding the fitness statistics and the analysis of variance (ANOVA).

Table 5. Summary of fit statistics.

Statistics	**Values**
Mean of response	9.7940
R^2	0.5137
Adjusted R^2	0.2219
RMSE	3.0596

The R^2 value of 0.5137 indicates that the model explains about 51.4% of the variability in the response variable. This moderate fit suggests that other unaccounted factors might influence the yield. The Adjusted R^2 of 0.2219, which is lower than R^2, accounts for the number of predictors in the model, indicating potential overfitting. An RMSE of 3.0596 shows the average deviation of predicted values from actual values, suggesting reasonable prediction accuracy.

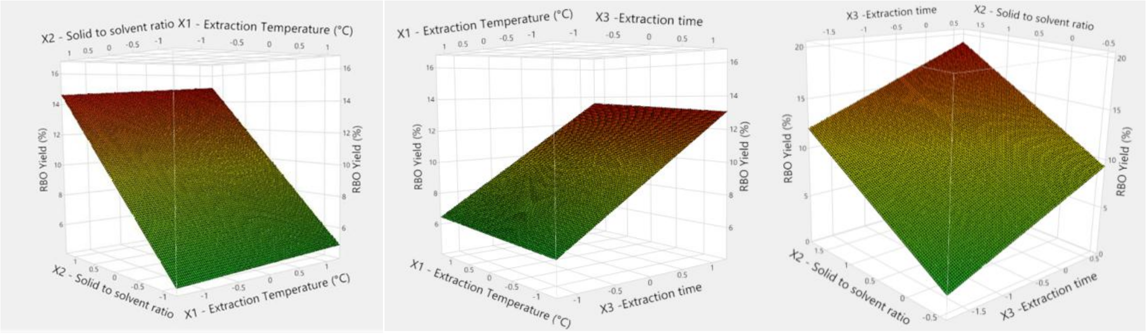
In addition, the ANOVA findings in Table 6 indicate that the model component is statistically significant, as evidenced by a p-value of 0.2706, which exceeds the threshold of 0.05. This further supports the appropriateness of the linear model in explaining the variability in the response variable. The linear model seems to be a suitable and accurate representation of the response surface in the examined extraction process, suggesting that the model is well-suited for the investigation. The fit statistics that are provided and the high correlation between the predicted and actual RBO yields support this Liu *et al.* (2006).

Table 6. Analysis of variance (ANOVA) for regression model

Source	**Sum of square (SS)**	**Df**	**Mean square (MS)**	**F ratio**	**P value**	**Remarks**
Model	49.4372	3	16.4791	1.7604	0.2706	Not significant
$X1$	0.2506	1	0.2506	0.0268	0.8764	Not significant
$X2$	33.1460	1	33.1461	3.5408	0.1186	Not significant
$X3$	16.0404	1	16.0404	1.7135	0.2475	Not significant
Lack of fit	13.6594	3	4.5531	0.2747		
Pure error	33.1460	2	16.5730			

The ANOVA results indicate that the regression model is not statistically significant, as the p-value (0.2706) is greater than 0.05. This suggests that the model does not adequately explain the variability in the response variable at the 95% confidence level. Therefore, the linear model is interpreted as exploratory for trend analysis rather than a robust predictive model. The results reveal that none of the individual predictors are statistically significant across all variables. The SS for $X1$ is 0.2506. It has a MS value of 0.2506 and a F ratio of 0.0268. The p-value of 0.8764 indicates that there is no significant relationship between temperature and the RBO yield. The SS for the $X2$ is 33.1460, with a MS of 33.1461 and a F ratio of 3.5408. The p-value of 0.1186 suggests that although the ratio has a relatively stronger impact compared to other variables, it is still not statistically significant at the 5% level. The SS for the $X3$ is 16.0404. The MS is 16.0404 and the F Ratio is 1.7135. The p-value of 0.2475 suggests that there is no statis0404tically significant relationship between extraction time and the outcome being predicted. The lack of fit analysis reveals that the SS is 13.6594, with a MS of 4.5531 and a F ratio of 0.2747. These results indicate that the residuals of the model do not depart considerably from the fitted values. This indicates that the model form is suitable, even if it does not have a high level of predictability. The pure error, with a SS value of 33.1460, represents the intrinsic variability in the data that cannot be accounted for by the model.

Interpretation of the 3D Response Surface Plots



(a) **(b)** **(c)**

Figure 8. 3D Surface plot of response with respect to (a) $\boldsymbol{X1}$ with $\boldsymbol{X2}$ (b) $\boldsymbol{X1}$ with $\boldsymbol{X3}$) (c) $\boldsymbol{X2}$ with $\boldsymbol{X3}$

Figure 8(a) illustrates that increasing the extraction temperature and finding the ideal solvent-bran ratio greatly improves the oil yield when isopropanol was used. As the temperature rises, the oil becomes more soluble in the solvent, leading to an enhancement in the rate of oil diffusion from the rice bran into the solvent. Nevertheless, there exists a specific limit where any more rise in temperature could potentially result in the deterioration of oil, thereby diminishing its productivity. The solvent-to-bran ratio must be ideal; a ratio that is too low will result in an insufficient amount of solvent to dissolve the oil, while a ratio that is too high would lead to declining returns owing to the saturation of the solvent. Figure 8(b) demonstrates that raising the extraction temperature and prolonging the extraction time often results in larger oil yields when employing isopropanol. Increasing the duration of the extraction process provides the solvent with a greater chance to deeply infiltrate the rice bran and effectively dissolve the oil. Figure 8(c) illustrates that the highest oil yield is achieved by utilizing an ideal solvent-bran ratio and a sufficient extraction time when isopropanol is employed. The comparison between predicted and actual yield is shown in Table 7.

Table 7. Predicted and actual comparison

Properties	**Experimental condition**			**Response**
	$X1$	$X2$	$X3$	Y, %
Predicted	70	1:7	5	14.04
Actual	70	1:7	5	14.87

The comparison between the predicted and actual yields under ideal circumstances reveals a strong correlation, with a mere 5.91% discrepancy. The strong agreement seen between the predicted and actual yields highlights the dependability of the optimized extraction parameters. According to Hasanah *et al.*, (2023), the extracted RBO yield was 18.52±0.04%, which deviates from the expected values by almost 0.81%. According to Sanket *et al.*, (2024), the predicted oil yield, denoted as 77.91% with a percentage error of -1.54%, is the anticipated quantity of oil that is forecasted to be obtained using mathematical models or predictive algorithms.

CONCLUSION

The study assessed the effectiveness of ethanol and isopropanol as solvents in the Soxhlet extraction of RBO, concluding that both solvents were successful with slight differences in the amount of oil extracted. Increasing the ratio of solvent to bran and extending the extraction duration improved the amount of oil obtained. These analyses confirmed the oil's composition and also revealed degradation caused by inadequate storage conditions. The stability of the shelf life was evaluated by quantifying the peroxide value, which indicated the occurrence of oxidative deterioration as time progressed. Minimising oxidative degradation can be achieved by optimising storage conditions. The study employed RSM to optimise the extraction temperature, duration, and solvent-bran ratio. The isopropanol at 70°C, with a ratio of 1:7 and a reaction time of 5 hrs, achieved the highest yield of 14.87% using this method. Under the same conditions, ethanol had a yield of 12.04%. The regression model was not statistically significant, according to the ANOVA results. This implies that, at the 95% confidence level, the response variable's variability cannot be sufficiently explained by the model.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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