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Optimization of the Synthesis of Oleyl Oleate from Olive-Based Fatty Acid by Response Surface Methodology

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Oleyl oleate, a liquid wax ester, was successfully synthesized via an esterification reaction between oleyl alcohol and oleic acid. Furthermore, the effects of essential reaction parameters on enhancing oleyl oleate formation were investigated. The reaction was optimized using the statistical approach of response surface methodology (RSM), relying on a 5-level, 4-variable central composite rotatable design (CCRD). This allowed for the evaluation of the interactive influence with regard to essential parameters in small-scale processing. The parameters studied included the reaction temperature, reaction time, molar ratios of the substrate (oleic acid:oleyl alcohol), and agitation speed, and the responses were oleyl oleate conversion. The maximum yield of 96.00% was accomplished with 3 hours 30 minutes reaction time, 53.9°C temperature, 172.9 rpm agitation speed, and a molar ratio of 2:24. The comparison between the anticipated and observed values demonstrated a strong alignment, suggesting that the empirical models formulated from RSM effectively capture the relationship between the factors as well as the outcome in the production process of oleyl oleate. Furthermore, the product was verified using spectroscopy methods, specifically Fourier transform infrared (FT-IR) as well as nuclear magnetic resonance (NMR). The absorption peak at 1712.18 cm^{-1} in the FT-IR spectrum demonstrated the characteristic ester carbonyl absorption in oleyl oleate. In addition, a peak at 173.2 ppm in the ^{13}C-NMR spectrum confirmed the presence of an ester group, further confirming the identity of the product.

Keywords: Oleyl oleate; wax ester; respond surface methodology (RSM); Fourier transform infrared (FT-IR); nuclear magnetic resonance (NMR)

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Fatty acids derived from vegetable oil have been widely employed in cosmetic formulations, with unsaturated fatty acids found in triglycerides proving to have the optimum cosmetic function. However, the problem is most vegetable oils are only partially soluble in aqueous solutions. Thus, their application in oil-based techniques in formulations is limited [1]. Therefore, more lipophilic derivatives should be produced by chemical modification of these oils by the synthesis of wax ester. Wax ester may be synthesized from multiple sources of vegetable oil, which includes olive oil, palm oil, and others, and it is widely used in the cosmetic industry for various reasons. In addition to synthetic methods, natural wax esters may also be obtained via sources, for instance, Jojoba oil and sperm whale oil, which are classified as fine chemicals. Wax ester is used as the main formulation in cosmetic production due to its properties, including non-toxic, great moisturizing at interfaces without oily texture during application to the surface of the skin and lipophilic characteristics [2]. Since wax ester has various functions that benefit the human body, it is also frequently used in the pharmaceutical, medicinal, and lubricant industries. The product can be produced from wax ester, such as creams, lipstick, powder, lotion, sunscreen, and others.

Wax esters are a type of ester that has a high molecular weight and are made up of long-chain alcohols and long-chain acids with carbon numbers between 12 and 32. Wax ester can be synthesized from the reaction between an organic acid and an alcohol with the acid catalyst presence to speed up the reaction. Other than that, it may be extracted from plants and animals, for instance, Jojoba oil, as well as sperm whales [3]. Non-soluble polymers in the cosmetics and pharmaceutical industries can be substituted with waxes that are natural, solvent-free, and cost-effective as an alternative [4]. Thus, the development of wax ester would have a great impact on the industry in terms of its usage and product development.

Wax esters are commonly used in industrial cosmetics and pharmaceuticals as the main component in their formulations, which has led to a high demand for wax esters production. As it is in high demand, the production of wax esters from natural sources, which include Jojoba oil and sperm whale oil, is limited due to its cost and availability, as well as the global ban on whale hunting [5]. Wax ester derivatives that are produced by modifying various types of vegetable oils with hydrophilic and lipophilic moieties have gotten a

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lot of attention among researchers. Thus, the researcher had to find an alternative to synthesize wax esters with low-cost materials as well as a shorter production time from vegetable oils such as olive oil, which has a similar property as natural wax esters [6].

Oleic acid was used as raw material from olive oil that further reacted with oleyl alcohol in the presence of n-hexane to produce oleyl oleate. Research indicates that oleic acid is associated with numerous benefits, including reducing the risk of cardiovascular diseases by increasing levels of high-density lipoprotein (HDL) while lowering levels of low-density lipoprotein (LDL) cholesterol [7]. Moreover, oleic acid has antioxidative properties, contributes to the reduction of cholesterol levels, and offers benefits such as longer shelf life in industrial applications [8]. The percentage of oleic acid in olive oil can range from around 55% to as high as 95%, depending on factors such as olive cultivar, maturity, and environmental conditions [9-11]. However, the optimization of reaction by conventional approach is laborious and time-consuming [12]. Due to the limitation, a new approach using the statistical method of central composite rotatable design (CCRD), response surface methodology (RSM), was used to optimize the reaction and produce a high yield of ester. CCRD allows for efficient exploration of the response surface by reducing the number of experimental runs needed while still providing accurate and reliable results. This design is particularly beneficial for optimizing processes involving multiple interacting factors, aiding in determining the optimal settings with regard to these factors to achieve the desired response [13]. The benefit of RSM is that it requires fewer experimental runs to obtain highly accurate data compared to synthesized data using the conventional method. Contrarily, the discovery of new lipophilic wax ester derivatives is expected to gain enhanced solubility, stability, and cosmeceutical qualities.

EXPERIMENTAL

Chemicals and Materials

Analytical-grade Ethanol, NaOH, n-hexane, Oleic acid, and Oleyl alcohol were obtained from Sigma-Aldrich (St. Louis, USA) and Merck (Darmstadt, Germany) for use in the study.

Synthesis of Wax Ester from Olive-based Fatty Acid

Oleic acid, a fatty acid derived from olive oil, was combined with a precise quantity of oleyl alcohol in a screw cap vial. Ratios ranging from 1:3 to 4:9 of oleic acid to alcohol, mixed with n-hexane as a solvent [14], were utilized. This mixture was then subjected to incubation at 40°C for 1 hour with continuous agitation at 150 rpm. The experimental parameters were adjusted according to RSM. Subsequently, the oleyl oleate's percentage conversion was determined through acid-base titration using 0.1 M NaOH to assess the remaining fatty acid content in the reaction mixture. The remaining unreacted oleic acid determines the percentage of oleyl oleate conversion.

Optimization of Reaction using Response Surface Methodology

A screw-capped vial was used to perform the reaction on a small scale. Table 1 shows the optimization process was carried out using RSM, Central Composite Rotatable Design (CCRD), which involved adjusting four main parameters: reaction time, agitation speed, reaction temperature, and substrate molar ratio (oleic acid: oleyl oleate). The analysis was conducted using Design Expert Version 13 software (Stat-Ease Inc., Minneapolis, MN, USA), which is used for statistics and data analysis [15].

Table 1. Design Matrix of the Actual Level for a Four-Factor CCRD for Oleyl Oleate.

	A	**B**	**C**	**D**	**Oleyl Oleate**
Standard	**Reaction Time**	**Reaction Temperature**	**Substrate Ratio**	**Agitation Speed**	**Predicted**
	(Hours)	(˚C)	ratio	rpm	(%)
1	1	40	1.5	100	70.98
2	5	40	1.5	100	76.07
3	1	60	1.5	100	74.36
4	5	60	1.5	100	84.29
5	1	40	3	100	82.14
6	5	40	3	100	74.24
7	1	60	3	100	84.86
8	5	60	3	100	81.79

9	1	40	1.5	200	74.6
10	5	40	1.5	200	87.78
11	1	60	1.5	200	76.9
12	5	60	1.5	200	94.91
13	1	40	3	200	80.52
14	5	40	3	200	80.69
15	1	60	3	200	82.16
16	5	60	3	200	87.16
17	7	50	2.25	150	83.13
18	3	30	2.25	150	78.26
19	3	70	2.25	150	88.12
20	3	50	0.75	150	79.65
21	3	50	3.75	150	83.07
22	3	50	2.25	50	76.23
23	3	50	2.25	250	85.23
24	3	50	2.25	150	93.65
25	3	50	2.25	150	93.65
26	3	50	2.25	150	93.65
27	3	50	2.25	150	93.65
28	3	50	2.25	150	93.65
29	3	50	2.25	150	94.72

Statistical and Graphical Analysis

The software Design Expert Version 13, created by State-Ease Inc. and labeled as "Statistics Made Easy," was employed to perform regression analyses, calculate the statistical significance of the models through ANOVA, and generate response surfaces. The software is based in Minneapolis, MN, USA.

Optimization of Reaction and Model Validation

The Design Expert software was employed to optimize the reaction parameters for each model using its numerical optimization capability. The software employs a measure known as "Desirability" (D), computed as the geometric mean with regard to all individual desirability factors ranging from 0 (least desirable) to 1 (most desirable). Should any of the responses lie outside their target range, the overall desirability function will register as 0. The designated conditions were subsequently employed for experimental runs, with the software utilized to forecast the reactions' percentage conversion.

Verification of Synthesize Wax Ester

Thin layer chromatography (TLC) was utilized throughout the process to confirm the completion of the reaction for the oleyl oleate product sample. Complementing the software, spectroscopic methods such as Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) were also utilized for sample analysis. These techniques enable the identification of different chemical compounds within the samples as well as the determination of their chemical structures.

Thin Layer Chromatography (TLC)

TLC served as an initial step prior to the confirmation of samples using FTIR and NMR. A sintered glass column (21 x 1.5 cm) was employed in this technique. The column was prepared by introducing a silica gel slurry in hexane to create a 10 cm high column. The sample solution dissolved in n-hexane was then pipetted into the column. The elution of the sample was carried out using hexane, ethyl acetate, and acetic acid as solvent systems, having a ratio of (8.5:20: 0.5, v/v). A single drop of the eluted sample was collected and subjected to TLC to isolate the sample by fraction. The purity of the oleyl oleate was subsequently extracted from the TLC paper for further analysis. Identification of oleyl oleate presence was accomplished by comparing the wax ester with an authentic standard.

Fourier Transform Infrared (FT-IR) Spectroscopy

The ester composition of the reaction mixture was assessed using an FT-IR spectrophotometer to identify its functional groups. Attenuated Total Reflectance (ATR) Infrared spectroscopy was employed to analyze the wax ester sample, and the absorption peaks were analyzed utilizing OPUS software.

Nuclear Magnetic Resonance (NMR)

Subsequently, the ester sample was subjected to NMR analysis, utilizing DMSO as the solvent, to elucidate the structure of the resulting product. The identification of the compound was based on the analysis of its chemical shifts in both the H-NMR and C-NMR spectra.

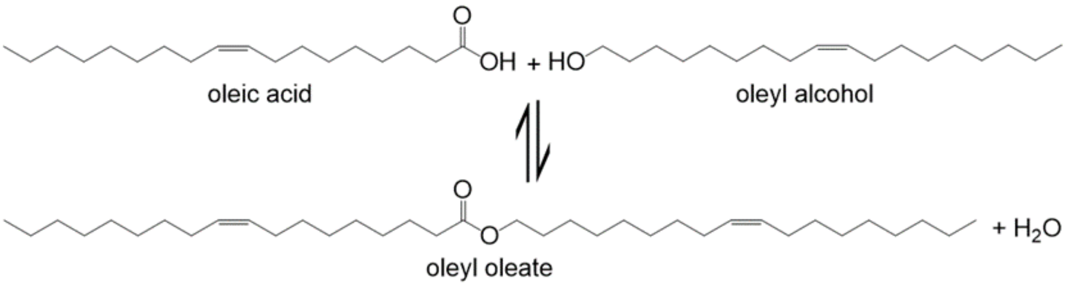
RESULTS AND DISCUSSION

Synthesis of Oleyl Oleate, a Wax Ester

Oleyl oleate was produced by an esterification reaction between oleic acid and oleyl alcohol. The use of n-hexane as a solvent helped dissolve the reactants and shift the equilibrium toward the desired product. Esterification was chosen over other synthetic methods due to its simplicity and minimal generation of byproducts, with only water being produced. Essentially, esterification can be seen as the opposite of hydrolysis. In this process, oleic acid acts as the acyl donor, reacting with oleyl alcohol as the nucleophile to form an ester. The reaction involves the addition of a proton, followed by the attack of the alcohol nucleophile, resulting in the formation of a tetrahedral intermediate. This intermediate then undergoes dehydration to complete the esterification reaction [16]. The esterification reaction between oleic acid and oleyl alcohol is illustrated in Scheme 1.

Model fitting and ANOVA by Response Surface Methodology (RSM)

The investigation of wax ester synthesis was conducted through ANOVA, as detailed in Table 2, affirming the model's accurate portrayal of the link between key variables and the outcome.



Scheme 1. Esterification Reaction between Oleic Acid and Oleyl Alcohol

Table 2. ANOVA for the Quadratic Model.

Sources	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Prob > F
Model	1427.42	14	101.96	49.32	0.0001*
Residual	28.94	14	2.07		
Lack of Fit	20.67	9	2.30	1.39	0.3752*
Pure Error	8.27	5	1.65		
Total	1456.36	28			

Table 3. R-Squared (R^2) Analysis of Quadratic Model.

Standard Deviation	1.44
Mean	83.76
R-squared (R^2)	0.9801
Adjusted-R-Squared (R^2)	0.9603
Predicted-R-Squared (R^2)	0.9002
Adequate Precision	23.149

The model's F-value of 49.32 indicates its significance, with a mere 0.01% likelihood of such a high F-value occurring because of random variation. P-values below 0.0500 suggest the significance with respect to the model terms, and in this case, the model's p-value of 0.0001 confirms its significance. Furthermore, the lack of fit F-value of 1.39 suggests that it was not significantly different from pure error. Here, the probability of a lack of fit F-value of this magnitude occurring due to noise was only 37.52%. This non-significant lack of fit is desirable as it signifies a good fit of the model. Table 3 displays the R-squared (R^2) analysis of the quadratic model.

The anticipated parameter values in Table 4, generated via Design Expert 13 software, are closely aligned with the observed values. Through the fitting of data into different models (linear, two factorials, quadratic, and cubic) as well as subsequent ANOVA analysis, it was determined that the optimal representation for the synthesis of oleyl oleate is a quadratic polynomial model.

Table 4. The Design Matrix of the Actual Experiments Carried Out for Developing the Model.

	A	**B**	**C**	**D**	**Oleyl Oleate**	**Oleyl Oleate**
Standard	**Reaction Time**	**Reaction Temperature**	**Substrate Ratio**	**Agitation Speed**	**Predicted**	**Actual**
	(Hours)	(˚C)	ratio	rpm	(%)	(%)
1	1	40	1.5	100	70.98	69.5
2	5	40	1.5	100	76.07	76.67
3	1	60	1.5	100	74.36	73.83
4	5	60	1.5	100	84.29	82.83
5	1	40	3	100	82.14	83
6	5	40	3	100	74.24	73
7	1	60	3	100	84.86	85
8	5	60	3	100	81.79	82.33
9	1	40	1.5	200	74.6	73.83
10	5	40	1.5	200	87.78	87.33
11	1	60	1.5	200	76.9	77.83
12	5	60	1.5	200	94.91	93.83
13	1	40	3	200	80.52	81.67
14	5	40	3	200	80.69	81
15	1	60	3	200	82.16	81.33
16	5	60	3	200	87.16	88.33
17	7	50	2.25	150	83.13	83.67
18	3	30	2.25	150	78.26	78.5
19	3	70	2.25	150	88.12	88.42
20	3	50	0.75	150	79.65	81.5

21	3	50	3.75	150	83.07	81.75
22	3	50	2.25	50	76.23	77.25
23	3	50	2.25	250	85.23	84.75
24	3	50	2.25	150	93.65	94.75
25	3	50	2.25	150	93.65	95
26	3	50	2.25	150	93.65	92
27	3	50	2.25	150	93.65	92.17
28	3	50	2.25	150	93.65	93.75
29	3	50	2.25	150	93.65	94.21

The forecasted R^2 value of 0.9002 showed a fair concurrence with the adjusted R^2 of 0.9603, as typically anticipated, with a difference of less than 0.2. Adequate precision evaluates the signal-to-noise ratio, in which a ratio exceeding 4 is favorable. With a ratio of 23.149, there is a sufficient signal, indicating the suitability of this model for exploring the design space.

Regression Analysis

Each series of coefficients resulting from the regression analysis of the data was subsequently employed to construct the model equation. These coefficient estimates signify the anticipated alteration in the response for each unit shift in factor value under the condition that all other factors remain unchanged. In an orthogonal design, the intercept represents the collective average response of all conducted trials. The coefficients, then, serve as alterations around this mean value based on the specified factor configurations. Negative coefficients indicate that the corresponding parameters exerted an adverse effect on the reaction [17].

In the quadratic model, it was found that the impact of reaction time, reaction temperature, substrate ratio, and agitation speed (A, B, C, D, AB, AD) was positively significant. The "prob > F" values below 0.0500 in Table 5 demonstrate the significance of the model terms. Moreover, the findings revealed that reaction time (A), reaction temperature (B), and agitation speed (D) had notable influences among the independent variables, with substrate ratio (C) showing the least significance. Additionally, all quadratic variables exhibited significant effects, as detailed in Table 5, depicting the significance of regression coefficients in the oleyl oleate synthesis process.

The final model equations encompassed all relevant terms. The equation, expressed in terms of coded factors, allows the prediction of the response based on each factor's specified levels.

Table 5. Values of Significance of Regression Coefficients for Synthesis of Oleyl Oleate (Quadratic Model).

Factor	Coefficient Estimate	Prob > F
Intercept	93.65	
A- Reaction Time	2.53	< 0.0001
B- Reaction Temperature	2.46	< 0.0001
C- Substrate Ratio	0.8546	0.0114
D- Agitation Speed	2.25	< 0.0001
AB	1.21	0.0047
AC	-3.25	< 0.0001
AD	2.02	< 0.0001
BC	-0.1669	0.6496
BD	-0.2706	0.464
CD	-1.31	0.0026
A^2	-3.89	< 0.0001
B^2	-2.61	< 0.0001
C^2	-3.07	< 0.0001
D^2	-3.23	< 0.0001

*significance at “Prob > F” less than 0.0500

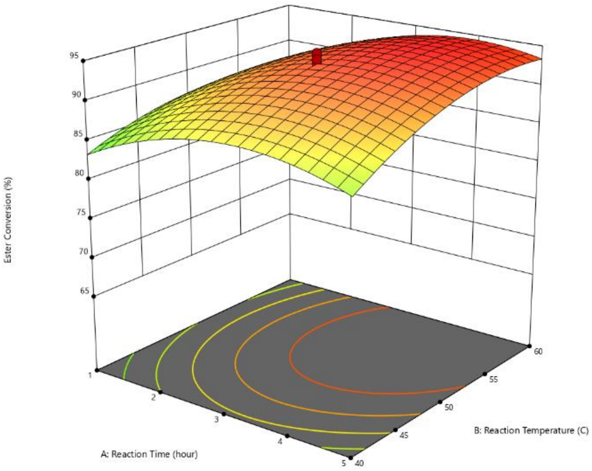


Figure 1. Response Surface Plot of Reaction Time versus Reaction Temperature (AB).

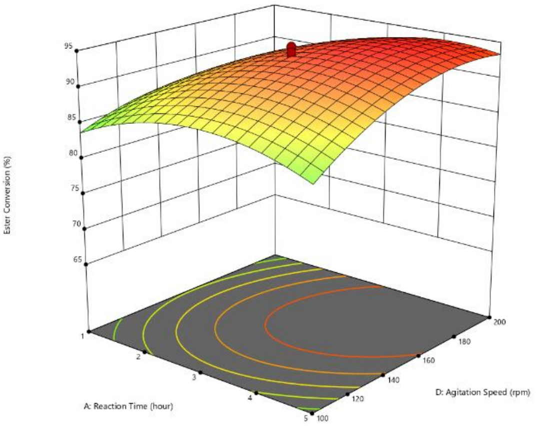


Figure 2. Response Surface Plots of Reaction Time versus Agitation Speed (AD).

High factor levels are coded as +1, while low levels are coded as -1, allowing for the comparison of factor coefficients to determine their relative influence. Hence, the final equation in terms of coded factors can be seen as below:

$$\text{Wax ester conversion} = 93.65 + 2.53A + 2.46B + 0.8546C + 2.25D + 1.21AB - 3.25AC + 2.02AD - 0.1669BC - 0.2706BD - 1.31CD - 3.89A^2 - 2.61B^2 - 3.07C^2 - 3.23D^2$$

in which A refers to the reaction time, B denotes the reaction temperature, C represents the substrate ratio, whereas D denotes the agitation speed.

Response Surface Analysis (Interactive Effect of Time, Temperature, and Agitation of Speed)

Reaction Time (A) versus Reaction Temperature (B)

The correlation between reaction duration and temperature notably affects the yield during oleyl oleate synthesis. Enhancing the reaction time from 3 to 4 hours within the range of 50-60°C enhances yields, reaching a maximum of 55-60°C, as depicted in Figure 1. However, beyond 60°C, the conversion diminishes, indicating a critical temperature threshold. Optimal temperature determination is pivotal, taking into account factors such as the melting point, which is influenced by structural characteristics. The reaction temperature strongly impacts the conversion of oleyl oleate, underscoring the necessity for meticulous temperature selection during esterification.

Reaction Time (A) versus Agitation Speed (D)

The reaction temperature and substrate ratio (mmol oleyl alcohol: mmol oleic acid) were maintained at their midpoint on the response surface plots to study the interaction between reaction time and temperature.

Figure 2 illustrates that the highest yields were achieved by increasing agitation speed from 140 rpm to 190 rpm and extending reaction time from 3 hours to 5 hours. Agitation speed is a crucial factor influencing molecular motion, with higher speeds promoting increased interactions between temperature and substrates. Additionally, it enhances the external mass transfer rates within the reaction mixture's bulk phase. However, elevated agitation speeds revealed a notable impact of reaction time on conversion. Beyond 180 rpm, the response began to decrease despite longer reaction times.

Reaction Temperature (B) versus Agitation Speed (D)

The duration of the reaction and the ratio of substrates (mmol oleyl alcohol: mmol oleic acid) were held constant at their central values, as depicted in the response surface plots for the interplay of reaction temperature and agitation speed. The wax ester's conversion percentage rose with the escalation of reaction temperature from 50°C to 60°C and agitation speed from 140 rpm to 200 rpm, illustrated in Figure 3. The reaction temperature has a more pronounced impact on ester conversion compared to agitation speed. This observation is supported by the fact that when agitation speed was increased from 100 to 200 rpm at a constant temperature of 40°C, the yield difference was merely 2%, whereas increasing the temperature from 40°C to 60°C at a fixed agitation speed of 200 rpm resulted in an 8% difference in yield.

Predicted vs Actual Analysis

Upon examination of the scatter plot illustrating the correlation between predicted and observed ester conversion values in Figure 4, it becomes evident that a strong agreement exists between the two sets of data. The points closely align with the line of perfect prediction, signifying a high level of accuracy in the predictive model. There is no observable consistent deviation from the ideal line across the entire range of values, indicating that neither over-predictions nor under-predictions are consistently present.

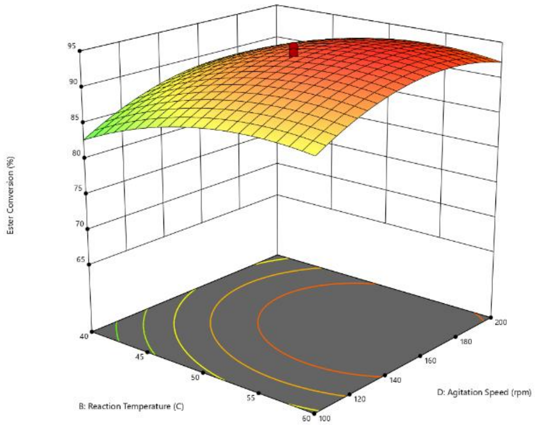


Figure 3. Response Surface Plots of Reaction Temperature versus Agitation Speed (BD).

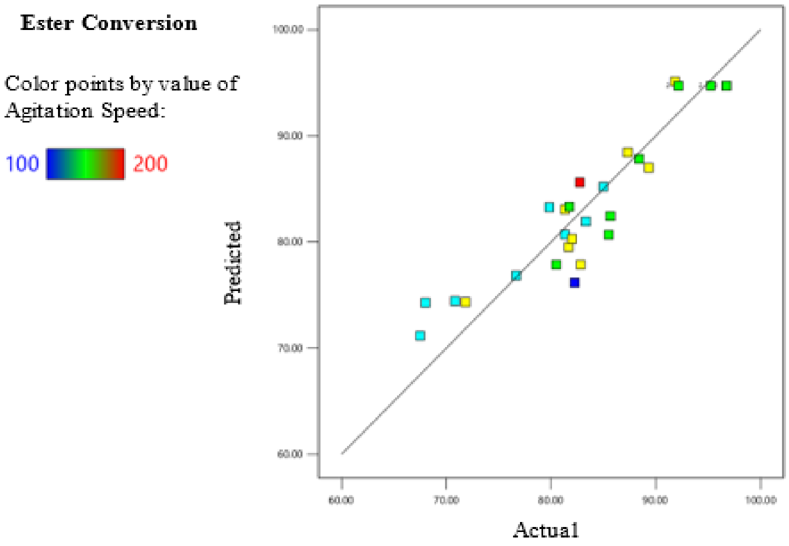


Figure 4. Predicted vs Actual of Ester Conversion.

The various colors denoting different agitation speeds do not seem to have a systematic impact on the accuracy of the predictions. The close match between predicted and observed values, along with the tight clustering of data points around the unity line, leads to the conclusion that the predictive model is valid for estimating ester conversion within the specified parameters. This model can be deemed sturdy and dependable for practical use within its relevant domain.

Optimum Condition

The RSM numerical optimization method was utilized to determine the best reaction parameters within the control factor range, accounting for the standard error (StdErr) within the model. Employing the desirability function offered an efficient means of attaining the optimal reaction conditions. By focusing on the goal of maximizing oleyl oleate conversion, a second-order quadratic polynomial model was employed to derive the optimal operational conditions through numerical RSM optimization.

The models indicate that a substantial conversion of 95.26% for oleyl oleate can be attained through simulation between two parameters at their respective actual factors. To validate the developed model and determine the best reaction conditions within the studied range, the optimization function of Design-Expert software was utilized. Numerous optimal operating variables and their corresponding predicted oleyl oleate conversions were generated. Subsequent experiments were conducted under these recommended conditions, and the resulting outcomes were compared with the predicted values for validation.

Table 6 displays the optimal conditions determined through experimentation and prediction. Comparing these values reveals a strong agreement, suggesting that the empirical model derived from RSM effectively describes the relationship between factors and response in oleyl oleate synthesis. The anticipated maximum yield of 95.26% was predicted to occur at a reaction time of 3 hours and 30 minutes, a temperature of 53.9°C, an agitation speed of 172.9 rpm, and a ratio of 2.24, as determined by Design Expert software's optimization function. Experimental validation yielded an actual yield of 96.00%, affirming the validity of the quadratic model and highlighting the efficacy of RSM in optimizing the synthesis of wax ester from olive-based fatty acids (oleyl oleate).

Verification of Synthesized Wax Ester by FTIR and NMR

Fourier Transform Infrared (FT-IR) Analysis

The evaluation of oleyl oleate's ester properties was observed through FT-IR analysis. FT-IR proves to be a valuable tool in discerning various chemical bonds within a molecule, providing an infrared absorption profile serving as a distinct molecular "fingerprint" [18].

Based on the findings in Figure 5, the presence of the ester carbonyl absorption at 1712.18 cm^{-1} indicates the formation of the ester bond in the oleyl oleate sample, resulting from the optimal conditions during the esterification of oleic acid with oleyl alcohol. Additionally, the FTIR analysis reveals other notable functional groups, such as the CH_2 and CH_3 bends of oleyl oleate, evidenced by absorption peaks at 1463.91 cm^{-1} and 1377.77 cm^{-1}, respectively. Furthermore, the CH- aliphatic stretching is observed at an absorption of 2922.65 cm^{-1}. Finally, the C-O stretching is detected within the range of 1055.42 – 1246.15 cm^{-1} [19]. Table 7 provides a summary of these vibration peaks.

Table 6. Predicted and Actual Values of Optimization Condition for Oleyl Oleate**.**

No.	Optimum Condition				Predicted Conversion (%)	Actual Conversion (%)
	A (Hours)	B (°C)	C (OA: OA)	D (rpm)		
1	3.5	53.9	2.24	172.9	95.26	96.00

Table 7. Summary of Vibration Peak.

Type of Vibration	Wavenumber (cm^{-1})	Intensity
-CH-aliphatic	2922.65	Strong
C=O ester bond	1712.18	Medium
C-O stretching	1055.42 – 1246.15	Weak
-CH_2-bend	1463.91	Medium
-CH_3-bend	1377.77	Weak

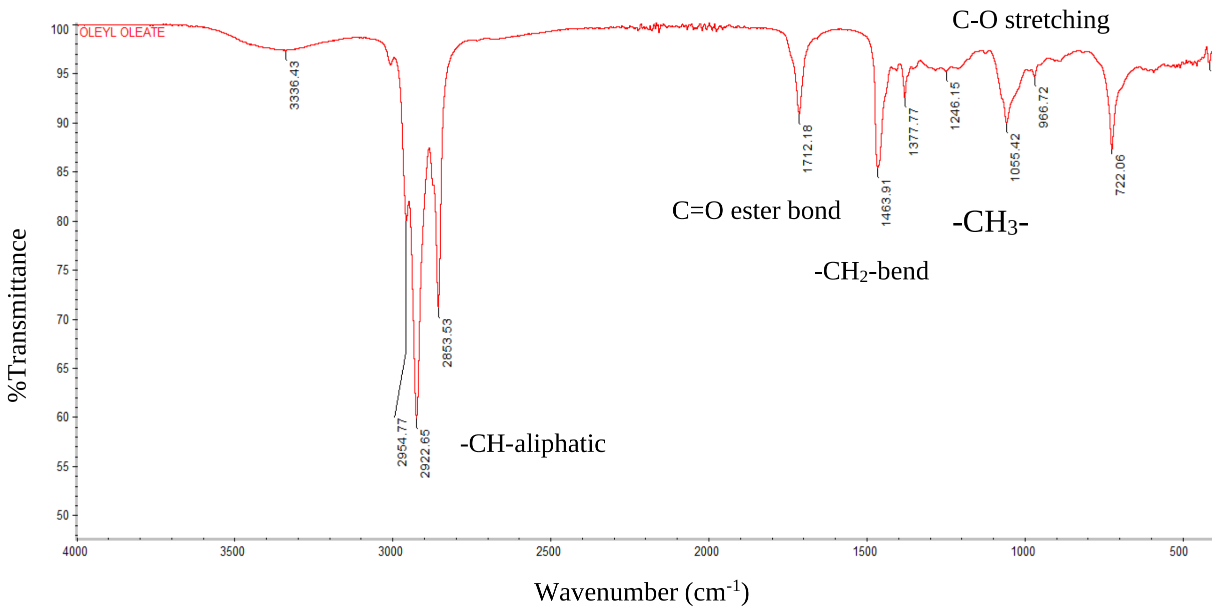
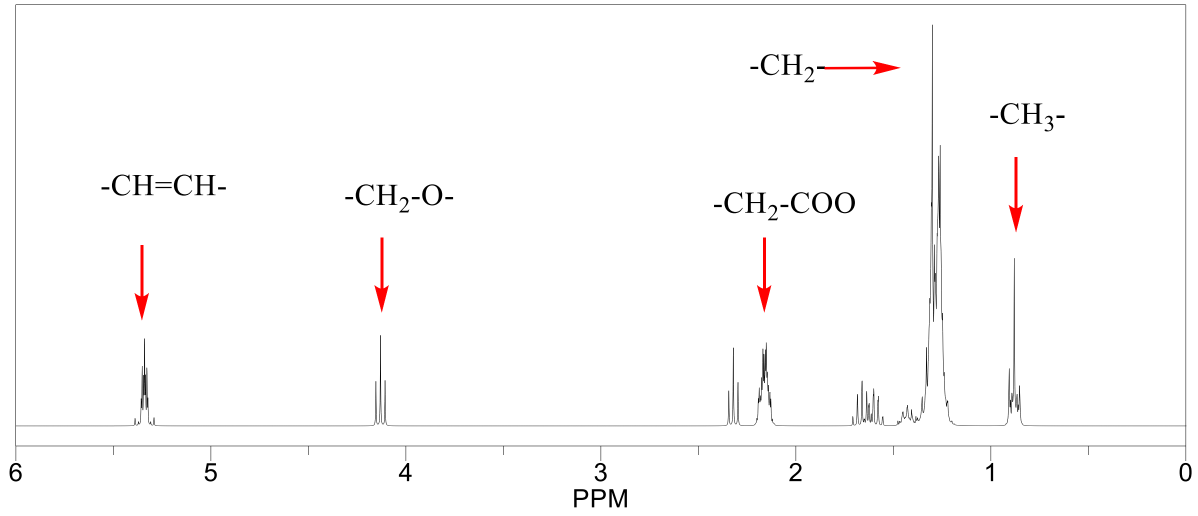


Figure 5. Infrared Absorption Spectrum of Oleyl Oleate.

^{1}H-NMR



^{13}C-NMR

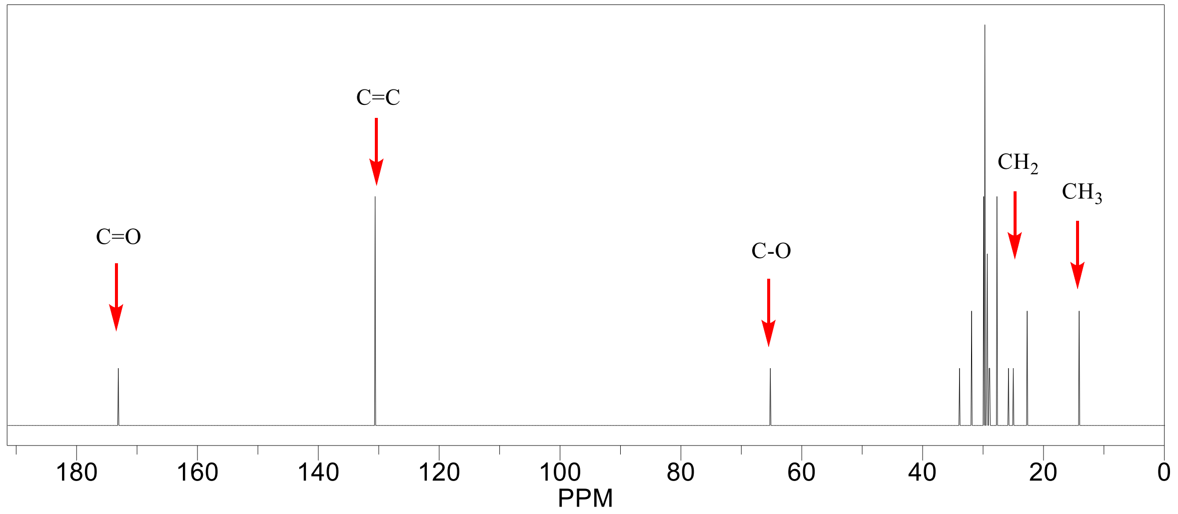


Figure 6. NMR Spectrum for ^{1}H-NMR and ^{13}C-NMR of Oleyl Oleate.

Nuclear Magnetic Resonance (NMR) Analysis

The ^{1}H-NMR spectrum of oleyl oleate in Figure 6 reveals a distinct 3-hydrogen triplet at 0.9 ppm, indicating the presence of a terminal methyl group. Additionally, two hydrogen methylene signals are observed in the range of 1.2 – 1.4 ppm. Notably, a methane hydrogen signal appears at 1.6 ppm, exhibiting a larger chemical shift compared to the previous methylene and methyl groups. Furthermore, the spectrum displays singlet and triplet signals for 3-hydrogen atoms between 2.1 – 2.5 ppm, suggesting that all hydrogens on a carbon adjacent to a carbonyl group exhibit absorption in the same magnetic field.

The identification of the $-CH_2-COO$ resonance at around 2.26 ppm in the ^{1}H NMR spectrum is crucial, as it signifies the successful completion of the esterification reactions. This notable chemical shift is a result of the deshielding effect caused by the electronegative oxygen atom bound to the same carbon. Furthermore, the ^{13}C-NMR spectrum of oleyl oleate (Figure 6) reveals important features. The peak at 14.1 ppm suggests the presence of aliphatic saturated carbon. A multitude of peaks falling between 22.76 and 64.59 ppm indicates the existence of aliphatic saturated and unsaturated carbons devoid of electronegative elements. Additionally, a peak at 130.6 ppm points to the presence of unsaturated carbon within the sample. The occurrence of an ester group is evidenced by the peak at 173.2 ppm, confirming the sample's ester nature. The principal signals representing functional groups in the ^{1}H-NMR and ^{13}C-NMR spectra of oleyl oleate are succinctly summarized in Table 8 and Table 9 [19].

CONCLUSION

This study focuses on enhancing the synthesis of wax ester (oleyl oleate) from olive-derived fatty acid through the application of RSM. The goal is to achieve a high yield through an economically efficient, catalyst-free process, which is crucial for industries aiming to create premium cosmetic products at reduced expenses. Through RSM optimization, a remarkable 96.00% conversion of oleyl oleate was successfully attained. The optimization process revealed that the duration of reaction, reaction temperature, and speed of agitation significantly influence ester conversion. In summary, this research underscores the value of RSM as a pivotal tool in optimizing wax ester production, catering to the escalating needs of the cosmetic industry while upholding cost-efficiency.

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Table 8. The Main Signals Present in ^{1}H-NMR Functional Groups of Oleyl Oleate.

1. Assignment	2. Chemical Shifts, ppm
3. -CH=CH-	4. 5.32
5. $-CH_2-COO$	6. 2.26
7. $-CH_2-O-$	8. 4.18
9. CH_2	10. 1.2-1.4
11. CH_3	12. 0.9

Table 9. The Main Signals Present in ^{13}C-NMR Functional Groups of Oleyl Oleate.

Assignment	Chemical Shifts, ppm
C=O	173.2
C=C	130.6
C-O	64.59
CH_2	32.82 22.76-29.98
CH_3	14.12

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