Enzymatic production of sustainable jojoba fatty alcohols. Biorefinery approach

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Abstract

To evaluate jojoba oil (JO) in high-added-value products, a new biorefinery strategy was used, consisting of enzymatic transesterification employing a branched 2-ethylhexanol (EH) chain alcohol. The suggested biorefinery approach utilizes an integrated process to produce jojobyl alcohols (JA) mixture (11-eicosenol, 13-docosenol, and 15-tetracosenol) as the principal product for pharmaceutical applications. Since it meets the European Biodiesel Standard EN 14214, the remaining fraction of fatty acid ethyl-hexyl esters (FAEHE) could be a potential alternative to traditional fuels.

Factorial design (FD) and response surface methodology (RSM) were used to investigate and improve the effects of variables such as temperature and catalyst concentration on the production of both fractions. The derived models can be used to estimate the best operating parameters for an up-scaled industrial process with the least number of tests possible, resulting in cost savings. However, from a technical perspective, the best feasible yield for the more valuable JA fraction should be achieved, using a catalyst concentration of 5.7%, a temperature of 63°C, and a 6:1 alcohol/oil molar ratio. Conversion rates of 63.5% and 36% for JA and FAEHE, respectively, could be produced under these conditions.

The crystallization method was used to separate JA from FAEHE. The MTT test was used to assess in vitro cell viability in HEK293T cells. The findings revealed that a 1 μmolL^{-1} oily liquid mixture of jojobyl alcohols components (cis-11-eicosenol, cis-13-docosenol, and cis-15-tetracosenol) had no influence on cell cycle progression, has no harmful impacts in the examined cells, and could be employed as a therapeutic compound.

The product preparation is a green engineering process that is clean, solvent-free, and uses a highly selective catalyst to reduce water and energy utilization, as well as the integrated process's downstream processing.

Introduction

The bioeconomy is a novel and critical paradigm for reducing our reliance on natural resources while also responding to the current environmental problems¹ The bioeconomy, according to the European Commission, is defined as the production of renewable biological resources and their conversion into value-added products such as food, feed, bio-based products, and bioenergy. Due to the employment of a wide range of scientific and industrial technologies, its sectors and industries have a high potential for innovation².

Governments and the chemical industry are currently working to encourage the substitution of fossil raw materials by renewable raw materials, the elimination of hazardous and polluting processes, the production of environmentally friendly (biodegradable) products, and the development of Green and Sustainable Chemistry, both for obtaining bioactive products and biofuels from renewable raw materials³.

In the search for green engineering processes, sustainable development has become the ideal key of the twenty-first century. The utilization of renewable raw materials such as vegetable oils is given a great deal of attention. Process integration could be utilized to keep prices down and help these processes compete in a market currently dominated by products made from other raw materials, such as petroleum and natural gas⁴.

Biorefinery is currently a well-accepted method for separating renewable raw materials into biobased process streams and, eventually, marketable chemicals and fuels. A successful biorefinery operation must achieve two strategic goals: displace nonrenewable raw materials (for environmental concerns, which are satisfied by biofuel production) and provide a financial incentive to promote a strong biorefining industry (economic goal, met by the production of high value chemicals). These objectives can be met at the same time by combining chemical and fuel production into a single operation⁵.

As both industrial and academic researchers recognize the environmentally benign or "green" approach, chemical processes that take environmental issues into account in the selection of reactants and reaction conditions are gaining in relevance. Green chemistry concepts aim to reduce, recycle, or eliminate the use of harmful compounds in chemistry by creating novel strategies to reduce human and environmental damage without limiting scientific development⁶.

Joboba (*Simmondsia chinensis*) is a perennial shrub in the Simmondsiaceae family that grows naturally in the deserts of the United States' southwest, Central and South America, South Africa, and many other countries. The seeds of the jojoba plant contain 45 to 55 percent of inedible oil–wax, which is primarily made up of straight chain fatty acid esters and straight chain fatty alcohols in the C20 to C44 range, with one double bond on either side of the ester bond⁷. JO and its derivatives are used in a variety of industries, including cosmetics, dietetic foods, animal feed, lubricants, and pharmaceuticals⁸. JO is also included as one of various biodiesel sources⁹. Its cultivation in the desert coastal parts of Spain (central-southern) and similar areas around the world would be beneficial, as it would allow these marginal areas to be exploited for industrial reasons, promoting local development.

The high viscosity and poor low-temperature properties of transesterified jojoba oil (TJO), which has a high cloud point (CP) and pour point (PP), are two of the most significant problems associated with the use of JO for biodiesel production¹⁰. Separating jojobyl alcohols as value-added products from fatty acids alkyl esters (FAAE) decreases FAAE viscosity and improves low-temperature characteristics making FAAE production more cost-effective¹¹.

Topical transdermal therapy of subdermal infections caused by herpes simplex viruses (HSV) and local dermal delivery of pharmacological substances for the treatment of various diseases are both possible with the liquid mixture of JA components and their formulations. JA are virustat that act as transdermal carriers for pharmacologically active drugs that act at or beneath dermal surfaces, delaying viral multiplication by limiting cellular penetration¹².

Immobilized lipases provide a number of benefits over typical transesterification catalysts, including the ability to perform under mild operating conditions, good selectivity, no significant side reactions, and high purity products. Furthermore, immobilized lipases are easily recoverable, and the final product is not contaminated, saving time and cost during the purification stage¹³.

In this context, we try to use jojoba oil as a feedstock for a clean integrated process that uses enzymatic transesterification of the oil with ramified-chain alcohol to improve the highly valued JA yield while decreasing purification steps and resulting in high quality products.

In this work and due to the importance of these esters in various industrial fields, a biorefinery approach was used to evaluate the integrated process of jojoba oil using an enzymatic transesterification to produce JA (jojobyl alcohols) as a main product and FAEHE (fatty acids ethyl hexyl esters) as co-products in order to overcome the economic problem. The effects of various variables on jojoba oil enzymatic alcoholysis have been investigated and optimized. Using factorial design and response surface methodology, the best values for the variables affecting the process were determined. Factorial design of experiments provides more information per experiment than unplanned approaches; it allows for the observation of interactions among experimental variables within the range studied, resulting in a better understanding of the process and, as a result, a reduction in research time and costs¹⁴. In order to achieve the hard criteria of low temperature characteristics of the esters, 2-ethyl-hexanol was employed as an alcohol. In HEK293T cells, the cytotoxic action of jojobyl alcohols was studied to see if they may be used therapeutically¹⁵.

Materials And Methods

Materials The fatty acid composition and main physico-chemical properties of the jojoba oil (JO) utilized in this study were determined using official methods of American Oil Chemists' Society (AOCS), as indicated in Table 1. Jojoba oil was provided by Jojoba Israel (Kibutz Hatzerin, Israel). Analytical reagent grade chemicals and solvents were employed throughout. A higher purity 2-ethylhexanol alcohol (99%) was provided by Sigma-Aldrich. Novozymes (Spain), S.A. kindly provided the catalyst, Novozyme 435 (Candida antarctica immobilized on acrylic resins).

Table 1: Composition and properties of jojoba oil

Composition	
Esters	Percent (%)
C34-C36	0.2
C38	6.6
C40	30.2
C42	50.9
C44	9.0
C46-C50	0.9
Properties	
Melting Point (°C)	9
Density (g/ml)	0.862
Iodine value (g/100 gr)	83
Acid Value (mg/g)	0.36
Viscosity (cSt)	26.6
Moisture (%)	0.03
Flash Point (°C)	225
CFPP(°C)	10
Oxidation Stability (h)	41.3

Table1. Characteristics of jojoba oil and fatty acids Composition

Equipment. A stirred batch three necked glass reactor with a volume of 500 cm³ was utilized to conduct all of the experiments. This reactor had temperature and speed control and was immersed in a thermostatically controlled water bath. The temperature was controlled with 1°C precision by a PID controller. A motor (IKA-labortechnik) controlled the speed of the mechanical stirrer. It was evaluated between 250 and 550 rpm, with 350 rpm being the optimal value for overcoming the external mass transfer constraint¹³.

Reaction procedure. The transesterification reaction of JO (Figure 1) was carried out according to Bouaid et al.^{9,16} procedure. The jojoba oil was introduced to reactor equipped with a reflux condenser. The catalyst and ethylhexanol alcohol were added once the temperature reached the specified point, and the impeller speed was kept at 350 rpm. At predetermined intervals, samples from the reaction mixtures were obtained and analyzed using gas chromatography (GC).

Reaction time. Reaction time is one of the most critical experimental variables that has a direct impact on economics and energy consumption¹⁷. To determine the reaction time, preliminary investigations were conducted. For 6 hours, the reaction kinetics were monitored. These findings indicate that the equilibrium was attained in 2 hours, with a 97% conversion rate. Increased reaction time had no effect on the yield of JA and FAEHE esters. As a result, the optimum reaction time for maximum yields was determined to be 2 hours. The reaction mixture was decanted and filtered after the reaction time had completed in order to separate the enzyme so that it could be recovered and reused later.

Separation of the jojobyl alcohols (JA). The jojobyl alcohols were separated using the method described by El boulifi et al.^{15,18}. The reaction mixture, 50g of transesterified jojoba oil (TJO), was combined with 100 mL of hot diethyl ether, then diluted with 300 mL of hexane. The mixture was vigorously agitated for 10 minutes at 25°C, then cooled to -18°C and left to rest for 24 hours. In a Buchner funnel, the solid jojobyl alcohols fraction was rapidly filtered under vacuum and washed with hexane. This method yielded a mixture of JA (C22:1:C24:1) in a ratio of 5.8:1. A rotary evaporator was used to evaporate the residual solution, and the process was repeated. Finally, in the second crystallization, the JA mixture (C20:1:C22:1:C24:1) in the ratio (5.4:10:1) was produced. The final solution was a mixture of fatty acid ethyl hexyl esters (FAEHE) with a minimum proportion of JA of less than 2.8%.

Analytical method

Gas chromatography (GC). Gas chromatography (GC/FID Hewlett-Packard 5890 series II and split-splitless injection system) was used to analyze jojoba oil and reaction products. The carrier gas was helium at a flow rate of 1mL/min. The separation program began with an initial oven temperature of 120°C, which was increased at 4°C/min to 160°C for 1 minute, followed by a 30°C/min ramp to 320°C, which was sustained for 20 minutes. The internal standard technique was utilized to determine the amount of chemical species. The other analytical operating conditions have been described in detail in a prior paper by Bouaid et al.¹³.

Non chromatographic analysis. The following procedures were used to measure the properties of the samples: acid value AV (UNE-EN 14,104), moisture content (Karl Fischer method, EN ISO12,937), and viscosity ν (ISO 3104). The oxidation stability of the FAEHE esters were determined according to Rancimat method, using Metrohm Rancimat 743 (Herisau, Switzerland). The cloud point (CP), pour point (PP), and cold filter plugging point (CFPP) of the esters were determined using an automatic analyzer (Cloud and Pour point measurements CPP 97-2), as described in ASTM D97 and ASTM D2500 methods.

Statistical analysis. The factorial design of experiments (FD) was used to investigate and optimize the synthesis of JA and FAEHE esters by enzymatic transesterification of jojoba oil. This study used a full two-level factorial design 2^2 (two factors at two levels each) amplified to Response Surface Methodology (RSM). This method's application requires the appropriate selection of response, factors, and levels.

The yield of JA and FAEHE ester was chosen as the response, Y . The factors were selected based on the process's chemical and economic standards. The reaction temperature, X_T , and the initial catalyst concentration, X_C , were chosen as the variables. The impeller speed was set at 350 rpm and the working pressure was set to 760 mmHg. The reaction was carried out based on the findings of a preliminary investigation, using an excess of alcohol, i.e. a molar ratio of alcohol to oil of 6:1, and the reaction period was set at 2 hours.

The levels were chosen based on the findings of a preliminary investigation, taking into account the experimental installation limitations as well as the working conditions limits for each chemical species. Because of these considerations, the upper temperature limit was set at 81.2°C to prevent enzymatic activity loss due to temperature. Temperatures below 40°C do not result in effective enzymatic activity¹⁹, hence yields achieved at these temperatures are insufficient for industrial purposes. As a result, the minimum temperature was set at 39°C. The levels of catalyst concentration were chosen based on preliminary experiments, usually between 2 and 8 wt. %²⁰. The levels selected were 2.2 and 7.8 wt.% of the total mass reaction. The amount of catalyst was gradually increased, and the ester yield was measured vs time.

The statistical analysis was conducted after these values were chosen. Table 2 shows the experimental matrix for the factorial design. The " ± 1 " coded factor levels in the dimensionless co-ordinate are shown in the first two columns, while the factor levels on a natural scale are shown in the next two. All of the experiments were carried out at random. Four experiments were carried out at the central point level, coded as '0', for experimental error estimates. The amount of JA and FAEHE produced could be expressed as a polynomial model due to the use of analysis and factorial design of experiments. As a result, we can plot the response, which is the theoretical product yield, as a function of the important factors and their interactions.

Table2. 2^2 Factorial experiment matrix: Experimental results.

Experiment	Coded design levels		Real values		Y _{JA} (%)	Y _{FAEHE} (%)
	X _T	X _C	T(°C)	C(%)		
1	-1	-1	45	3	51	46
2	+1	-1	75	3	43	54
3	-1	+1	45	7	49	47
4	+1	+1	75	7	64	34
5	0	0	60	5	59	38
6	0	0	60	5	62	35
7	0	0	60	5	60	37
8	0	0	60	5	59	41

Results And Discussion

Linear stage. In this study, a 2^2 factorial design was used, with four central points added to assess the experimental error. Table 2 displays the results obtained. These experimental values were statistically analyzed, and the statistically significant effects and interactions for two variables were calculated. Tables 3 and 4 show the results of the statistical significance tests.

Table 3. 2^2 Factorial design for linear model: Statistical analyses for jojobyl alcohols(JA).

Response: Yield of ester after 2h of reaction		
Number of runs: 4		
Freedom degrees: 3		
Results of statistical analysis		
Y = 52		
Interactions		
$X_T = 3.5$	$X_C = 9.5$	$X_{TC} = 11.5$
Significance test		
Confidence level: 95%		
$Y_C = 59$	$S = 2.5$	$t = 2.35$
Confidence range: ± 3		
Main Effects and interactions: $X_C(+), X_T(+), X_{TC}(+)$,		
Curvature: $C = Y - Y_C = -7.5$		
Curvature effect: $I_{CC} = \pm 4$		
Response equation		
$Y = 52 + 4.75X_C + 1.75X_T + 5.75 X_{TC}$		$r=0.96$

Table 4. 2^2 Factorial design linear model: Statistical analysis for fatty acids ethyl hexyl esters (FAEHE).

Response: Yield of ester after 2h of reaction		
Number of runs: 4		
Freedom degrees: 3		
Results of statistical analysis		
Y = 45		
Interactions		
$X_T = -2.5$	$X_C = -9.5$	$X_{TC} = -10.5$
Significance test		
Confidence level: 95%		
$Y_C = 38$	$S = 2.25$	$t = 2.35$
Confidence range: ± 2.78		
Main Effects and interactions: $X_C(-), X_T(-), X_{TC}(-)$		
Curvature: $C = Y - Y_C = 7.5$		
Curvature effect: $I_{CC} = \pm 4$		
Responses equation		
$Y = 45 - 4.75X_C - 1.25X_T - 5.25X_{TC}$		$r=0.90$

Multiple regression analysis was used to fit the temperature (X_T) and catalyst concentration (X_C) effects, as well as their interactions, to a linear model. For the main significant effects and interactions, the response function is as follows:

Equation (1)

$$Y_{JA} = 52 + 4.75X_C + 1.75X_T + 5.75X_{TC} \quad r=0.96$$

Equation (2)

$$Y_{FAEHE} = 45 - 4.75X_C - 1.25X_T - 5.25X_{TC} \quad r=0.90$$

The catalyst concentration is the most positive significant factor, as shown in the statistical study. The statistical analysis of the experimental results further shows that both the JA and FAEHE processes have a considerable curvature effect (Table 3 and Table 4). As a result, a different approach was required, involving fitting the data to a second-order model.

Non-linear stage. Since of the importance of the curvature effect observed in the linear stage, a second-order model is necessary for the JA and FAEHE processes according to the central composite design methodology, the experiments have been amplified using a Response Surface Methodology. For the two significant factors, reaction temperature and catalyst concentration, additional experimental points (star points) had to be included into the two-level factorial design. Tables 2 and 5 illustrate the full central composite design, which includes factorial points, centre-points, and star points adapted from Box and Wilson²¹. As demonstrated in Eq. (3), the appropriate model is the entire quadratic surface between the response and the factors:

Equation (3)

$$Y = a_0 + \sum_{k=1}^2 a_k X_k + \sum_{k=1}^2 a_{kk} X_k^2 + \sum_{k \neq j}^2 a_{kj} X_k X_j$$

Table5.- Experimental results of the star points.

Experiment	Coded design levels		Real values		$Y_{JA}(\%)$	$Y_{FAEHE}(\%)$
	X_T	X_C	T(°C)	C(%)		
9	$-\alpha$	0	38.85	5	48.0	49.0
10	$+\alpha$	0	81.15	5	51.0	45.0
11	0	$-\alpha$	60	2.18	48.0	48.0
12	0	$+\alpha$	60	7.82	47.0	49.0

To build a central composite design, four extra runs, named star points and coded $\pm\alpha$, were added to the 2^2 factorial design and are summarized in table 5, where α , the distance from the origin to the star point, is given by $\alpha=2^{n/4}$, in the design, $\alpha= 1.41$. Multiple regression analysis was used to determine the coefficients of Eq. (3). Regardless of their significance levels, all independent variables and their interactions are included in this analysis. The following statistical model can be used to express the best-fitting response surfaces:

$$\text{Equation (4): } Y_{JA} = 59.25 + 2.2X_C + 1.4X_T + 5.75X_{TC} - 5.1X_C^2 - 4X_T^2 \quad r=0.97$$

$$\text{Equation (5): } Y_{FAEHE} = 37.75 - 2.1X_C + 1.33X_T - 5.25X_{TC} + 4.56X_C^2 + 4.1X_T^2 \quad r=0.95$$

Coded levels were used to create the statistical model. Equations (4) and (5) can be visualized as dimensional surface plots (see Figs. 2 (a&b) and 3 (a&b)), indicating the estimated yields for JAs and FAEHEs over the investigated range of initial catalyst concentration and temperature.

The effect of catalyst concentration and reaction temperature on JA and FAEHE yields will be analyzed in the following sections. Statistical models will be used to evaluate the effect of the main factors and interactions.

Influence of the initial catalyst concentration. For effective industrial applications, the amount of enzyme employed in the process is critical²². By increasing the biocatalyst amount from 2.18 %(- α) to 7.82%(+ α), the effect of the biocatalyst quantity on the reaction yield is examined. Based on the statistical analysis, the initial catalyst concentration is the most important factor affecting the transesterification process for JA and FAEHE synthesis within the experimental range. It has a positive effect on the JA response but a negative effect on the FAEHE response. However, when the lipase amount exceeds 5%, the percentage of JA yield is slightly lowered (Fig. 4). This could be due to the presence of a large amount of enzyme, the active site cannot be exposed to the substrates, and numerous enzyme molecules clump together²³.

Influence of temperature. The production of JA increased when the reaction temperature was raised, however after reaching the optimum temperature, the yield of JA began to decline (Fig. 4). In the examined range (39–81.2°C), the influence of temperature is statistically significant for both the linear and non-linear models. This effect has a positive effect on the process. Temperature enhances the solubility of alcohol in oil, improves catalyst particle dispersion in liquid media, and improves mass transfer between reactants²⁴ which could explain a positive effect on conversion. The product yield JA began to decline as the temperature rose beyond 60°C, probably because higher temperatures speed the inactivation of the enzyme. This implies that, while temperature has a significant impact on the process, it is not as significant as catalyst concentration. Working temperatures in the 60–65°C range, on the other hand, are recommended to improve operational stability.

Influence of (T–C) interaction. All of the factors employed in the design have binary influences according to the linear model. The interaction of the two most important effects, temperature and catalyst

concentration (T-C), is considerable and has a positive influence on the JA process. This effect is stronger on JA production than on FAEHE production.

Analysis of response: JA and FAEHE yields. The yield was determined as the weight percentage of the products with respect to the amount of vegetable oil (jojoba oil) fed into the reactor. The corresponding yields for JA and FAEHE relate to the concentration of JA and FAEHE (wt.%) in the entire reaction mixture; numerical values were measured using capillary gas chromatography.

The statistical model's significance can be seen by plotting the response (JA and FAEHE conversions) as a function of the two variables (enzyme concentration and temperature). Figures 2 (a&b) and 3 (a&b) show the surface and contour plots of JA and FAEHE yields vs catalyst concentration and temperature derived using individual experimental data. When it comes to JA, a comparison of these plots reveals that the highest yield ($Y_{JA}=60-64\%$) is obtained by utilizing a high enzyme concentration (5.4-6.6%) and working at a temperature of 63-73 °C. However, from a technical perspective, the best feasible yield for the more valuable JA fraction should be obtained, which requires a catalyst concentration of 5.7% and a temperature of 63°C. Conversion rates of 63.5% and 36% for JA and FAEHE, respectively, could be achieved under these conditions.

The residual distribution, defined as the difference between estimated and observed values, over the observed values for the two responses tested, JA and FAEHE yields, is plotted in Figure 5 (a&b). In both situations, the fit quality is good because the total of residual errors is close to zero and the residual distribution follows a very modest trend with regard to the predicted values. For the JA and FAEHE yields, all residuals are less than 6% and 5%, respectively, indicating that the models adequately describe the JA and FAEHE yields over the experimental range investigated.

Separation of JA from FAEHE. The fatty acid component of jojoba oil (JO) is predominantly made up of eicosenoic, erucic, and oleic acids, with the alcohol component primarily made up of cis-11-eicosenol, cis-13-docosenol, and cis-15-tetracosenol. Alcoholysis produces a product that contains a mixture of JA and FAEHE. The JA are substantially less soluble than their FAEHE counterparts, a mixture containing both compounds can be partially separated. As a result, the crystallization process can be a useful tool for correctly separating jojobyl alcohols from esters. Crystallization operations were performed on the transesterified reaction obtained under the optimal working conditions predicted by the quadratic model. The solvents employed to crystallize and isolate the product of interest (JA) were a 3:1 ratio hexane/diethyl ether binary system. Using this solvent system, more than 95% of the jojobyl alcohols were recovered during the transesterification reaction.

Quality control of FAEHE as Biodiesel. Table 6 illustrates experimental results for some of the most relevant FAEHE quality parameters (acid value, viscosity, cloud point, pour point, cold filter plugging point, and oxidative stability). Except for the viscosity value criteria, the values were in accordance with the European Biodiesel Standard EN14214.

Table 6. Quality control of fatty acids ethyl hexyl ester (FAEHE) compared to EN 14214

Property	FAEHE	EU Standard EN14214
Viscosity (cSt)	15.50	3.50 - 5.00
Water content (%)	0.03	Max 0.05
Density at 15°C(g/cm ³)	0.886	Min 0.86-Max 0.90
Acid value (mg KOH/g)	0.15	Max. 0.5
Ester contents (wt.%)	97.2	Min. 96.5 % (m/m)
Oxidation stability (h)	32.5	> 8h
Cold filter plugging point (°C)	-21	Summer ≤ 0 Winter ≤ -10
Cloud point (°C)	-18	a
Pour point (°C)	-22	a

^a Not specified. EN 14214 uses time and location dependent values for the cold filter plugging point (CFPP) instead.

Kinematic viscosity. The kinematic viscosity measurement of FAEHE revealed that ethylhexanolysis produced an ester with a higher kinematic viscosity (15.53 cSt). This could be owing to the alcohol's chain length and branching, which raises the viscosity.

Low-temperature properties. Three typical measures, cloud point (CP), pour point (PP), and cold filter plugging point (CFPP), are used to determine the cold flow properties and low-temperature operability of fatty acid ethyl-hexyl ester (FAEHE). CFPP, as compared to CP or PP, is generally considered to be a more trustworthy indicator of low temperature operability²⁵.

Table 6 reveals that the fatty acid ethyl-hexyl ester has good characteristics at low temperatures, with CP, PP, and CFPP values of -16°C, -21°C, and -20°C, respectively. According to the findings, producing FAEHE from JO and utilizing 2-ethylhexanol as an alcohol improved cold flow qualities in terms of CP, PP, and CFPP when compared to using short and linear chain alcohols like methanol, ethanol, and butanol as alcohols in the transesterification reaction²⁶.

Oxidative stability. The Rancimat results are shown in Table 6. In comparison to the results obtained by El boulifi et al.,¹⁸ using methanol, ethanol, and butanol as transesterification alcohols, the FAEHE produced from jojoba oil and using 2-ethylhexanol as alcohol in the transesterification process showed good oxidative stability close to 30h according to the rancimat test. The material, on the other hand, can be utilized directly as a biofuel to provide energy for the biorefinery, making the process more efficient and environmentally friendly. Even for use as biodiesel, numerous treatments, as mentioned by Nestor et al.,²⁷ might easily improve the viscosity.

Jojoba Oil biorefinery. In a typical jojoba oil-based biorefinery, figure 6 describes various processing steps for the synthesis of jojobyl alcohols (JA) and fatty acid ethyl hexyl esters (FAEHE).

After the transesterification reaction was completed, the sample was decanted and filtered to remove the enzyme for later reuse, as shown in Fig. 6. After the enzyme was separated, the excess alcohol in the transesterified jojoba oil phase was eliminated and recovered. The jojobyl alcohols (11-eicosenol, 13-docosenol, and 15-tetracosenol) were then separated from the fatty acid ethyl hexyl esters using two crystallization steps.

Activity of Jojobyl alcohols. The MTT test was used to assess cell viability in HEK293T cells in vitro. The results revealed that jojobyl alcohols were cytotoxic at concentrations of $10\ \mu\text{molL}^{-1}$ and $100\ \mu\text{molL}^{-1}$, but had no effect at $1\ \mu\text{molL}^{-1}$. As a result, a concentration of $1\ \mu\text{molL}^{-1}$ has no influence on cell viability, has no harmful effects in the cells examined, and might be employed as a therapeutic agent as described in more detail in our prior paper¹⁵.

Conclusions

The purpose of this study was to use a biorefinery approach to valorize jojoba oil, focusing on the synthesis of the high-value-added product jojobyl alcohols (11-eicosenol, 13-docosenol and 15-tetracosenol). Using Novozyme as a catalyst, the effects of enzyme concentration and temperature on JA and FAEHE production were investigated and optimized. Under optimal conditions, the enzyme was more resistant to denaturation by 2-ethylhexanol.

According to this investigation, the highest yield jojobyl alcohols ($Y_{JA}=63.5\%$) and fatty acid ethyl hexyl esters ($Y_{FAEHE}=36\%$) could be achieved from a technical perspective. The produced FAEHE fraction meets the requirements of the European Union Biodiesel Standard EN 14214 and can be utilized as a diesel fuel alternative with a high added value, making the process both commercially and environmentally viable. Furthermore, immobilized lipases are easily recoverable, and the final product is not contaminated, saving time and resources during the purifying process. As a result, using jojoba oil as a renewable feedstock is critical for constructing an integrated and self-sustaining biorefinery.

At $1\ \mu\text{molL}^{-1}$, the mixture of jojobyl alcohols components (cis-11-eicosenol, cis-13-docosenol, and cis-15-tetracosenol) had no cytotoxic effect and might be employed as a therapeutic agent, according to the cell viability data. Jojoba oil has the potential to be a key component of a circular bioeconomy framework.

Abbreviations

JA: Jojobyl alcohols

FAEHE: fatty acid ethyl hexyl esters

C catalyst concentration, %

T	reaction temperature, °C
MR	Molar ratio
TC	temperature-catalyst concentration interaction
X_i	level of factor i
Y	ester conversion, %
n	number of factors in a factorial design
r	correlation coefficient
t	Student's t value
s	standard deviation
1.	distance from origin to star point in a central composite design
Y	mean response for the factorial design
Y_c	mean centrepointh response
a_0	intercept
a_k	first-order model coefficient
a_{kk}	quadratic coefficient for the i^{th} variable
a_{kj}	interaction coefficients for the interaction of variables k and j
X_k	independent variables

Declarations

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Authors' contributions:

Natalia Tardón: Validation, Formal analysis, Investigation, Resources. **Olivia Hurtadob:** Conceptualization, Investigation Methodology, Writing - Review & Editing, Visualization,. **Abderrahim Bouaid:**

Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision. **Jorge Mario Marchetti**: funding, writing – review and editing

Conflicts of interests:

The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

Availability of data and materials:

All data generated or analysed during this study are included in this published article

Additional information

Ethical approval

There was no animal experiment in this manuscript.

Consent to participate and publish

All co-authors have seen and approved the manuscript and have agreed to its submissions for publication

References

1. Ación Fernández, F.G., Reis, A., Wijffels, R., Barbosa, M., Verdelho, V., Llamas B. The role of microalgae in the bioeconomy. *New Biotechnology*. 99–107 (2001).
2. European Commission. *Innovating for Sustainable Growth: A bioeconomy for Europe*. Brussels, (2012).
3. Project: Horizon 2020 multi-actor projects - EIP-AGRI Brochure 28 September 2017. <https://ec.europa.eu/programmes/horizon2020/en/h2020-section/bioeconomy> (accessed 04 May 2021).
4. Pandey, A., Soccol, C.R., Mitchell, D.K.,. New developments in solid state fermentation: 1-Bioprocesses and products. *Process Biochem.* **35**, 1153–1169 (2000).
5. Janssen, R., Turhollow, A.F., Rutz, D., Mergner, R. Production facilities for second-generation biofuels in the USA and the EU – current status and future perspectives. *Biofuels, Bioprod. Bioref.* **7**, 647–665 (2013).
6. Anastas, P.T., Warner, J.C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York (1998).
7. Wisniak J, *The Chemistry and Technology of Jojoba Oil*, first ed. Amer. Oil Chem. Soc., Champaign (1987).

8. Shahidi, F. *Bailey's Industrial Oil and Fat Products*. Volume 1, Edible Oils and Fat products: Chemistry, Properties, and Health effects, John Wiley & Sons, Inc., Hoboken, New Jersey, USA (2005).
9. Bouaid, A., Bajo, L., Martinez, M., Aracil, J. Optimization of biodiesel production from jojoba oil. *Process Saf. Environ. Prot.* **85**, 378–382 (2007).
10. Sánchez, M., Avhad, M.R., Martinez, M., Aracil, J., Marchetti, J. Jojoba oil: A state of the art review and future prospects. *Energy Conversion and Management*. **129**, 293–304 (2016).
11. Avhad, M.R., Sánchez, M., Peña, E. Bouaid, A., Martinez, M., Aracil, J., Marchetti, J. Renewable production of value-added jojobyl alcohols and biodiesel using a naturally-derived heterogeneous green catalyst. *Fuel*. **179**, 332–338 (2016).
12. Verbiscar AJ, Topical transdermal treatments. United States Patent US 6858232 (B2). Feb 22 (2005).
13. Bouaid, A., Aparecio, J., Martinez, M., Aracil, J. Synthesis of a green biosolvent: Isopropyl esters: A statistical approach. *Enzyme and Microbial Technology*. **41(43)**, 533–538 (2007).
14. Box, J., Wilson, W. Central composites design. *J R Stat Soc* **1**, 1–35 (1951).
15. Acherki, H., Hurtado, O., Bouaid, A., 2021. Sustainable production of jojobyl alcohols and cell viability study. *Biofuels, Biopro. Bioref.* **16(2)**, 451–459 (2022).
16. Bouaid, A., Acherki, H., García, A., Martinez, M., Aracil, J. Enzymatic butanolysis of coconut oil. Biorefinery approach *Fuel*, **209**, 141–149 (2017).
17. Balat, M., Balat, H. Progress in biodiesel processing. *Appl. Energy*. **87**, 1815–1835 (2010).
18. El Boulifi, N., Sánchez, M., Martinez, M., Aracil, J. Fatty acid alkyl esters and monounsaturated alcohols production from jojoba oil using short-chain alcohols for biorefinery concepts. *Industrial Crops and Products*. **69**, 244–250 (2015).
19. Razack, S.A., Duraiarasan, S. Response surface methodology assisted biodiesel production from waste cooking oil using encapsulated mixed enzyme. *Waste Management*. **74**, 98–104 (2016).
20. Garcia, T., Sanchez, N., Martinez, M., Aracil, J. Enzymatic synthesis offatty esters. Part II. Optimization studies. *Enzyme Microb Technol.* **25**, 591–597 (1999).
21. Jiang, Y., Liu, X., Chen, Y., Zhou, L., He, Y., Ma, L., Gao, J. Pickering emulsion stabilized by lipase-containing periodic mesoporous organosilica particles: a robust biocatalyst system for biodiesel production. *Bioresour. Technol.* **153**, 278–283 (2014).
22. Calero, J., Verdugo, C., Luna, D., Sancho, E.D., Luna, C., Posadillo, A., Bautista, F.M., Romero, A.A. Selective ethanolysis of sunflower oil with Lipozyme RM IM an immobilised *Rhizo mucor miehei* lipase, to obtain a biodiesel like biofuel, which avoids glycerol production through the monoglyceride formation. *New Biotechnol.* **31**, 596–601 (2014).
23. Lee, H.V., Yunus, R., Juan, J.C., Taufiq-Yap, Y.H. Process optimization design for jatropha-based biodiesel production using response surface methodology. *Fuel Process. Technol.* **92**, 2420–2428 (2011).
24. Park, J.Y., Kim, D.K., Lee, J.P., Park, S.C., Kim, Y.J., Lee, J.S. Blending effects of biodiesels on oxidation stability and low temperature flow properties. *Bioresour. Technol.* **99**, 1196–1203 (2008).

25. Nestor, U., Soriano, Jr., Migo, V.P., Matsumura, M. Ozonized vegetable oil as pour point depressant for neat biodiesel. Fuel. 85(1), 25–31 (2006).

Figures

Figure 1

Scheme of the transesterification of jojoba oil

Figure 2

Response surface (a) and contour plot (b). of yield us catalyst concentration and temperature for JA, time, $t=2h$.

Figure 3

Response surface (a) and contour plot (b). of yield us catalyst concentration and temperature for FAEHE, reaction time, t=2h.

Figure 4

Residual plots of JA and FAEHE yield for the second order model. (a): JA and (b): FAEHE

Figure 5

Figure 6
A schematic representation of jojobyl alcohols (JA) and fatty acids ethyl hexyl esters (FAEHE) from the jojoba oil biorefinery