



# Optimizing the Conversion of Bio-Oil from *Haematococcus pluvialis* to Fatty Acid Methyl Esters

C. Sanjurjo<sup>1</sup> · E. Rodríguez<sup>1</sup> · M. Bartolomé<sup>2</sup> · R. González<sup>2</sup> · A. Hernández Battez<sup>1</sup>

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## Abstract

*Haematococcus pluvialis* microalgae have emerged as a prevalent source of antioxidants in cosmetics and nutritional products. Additionally, numerous researchers have posited the potential of this microalgae to produce fatty acid methyl esters (FAME). Nevertheless, the optimization of the production of FAME from *H. pluvialis* oil has not been investigated. In this study, the transesterification reaction of *H. pluvialis* bio-oil was optimized using the response surface methodology, resulting in optimal experimental conditions for an oil to methanol ratio of 1:4.17, at a temperature of 80 °C, with a reaction time of 47 min. The resulting FAME was found to not comply with the biodiesel standard in terms of the content of polyunsaturated fatty acids (6.02%), as well as kinematic viscosity (7.02 mm<sup>2</sup>/s). Further study is required to reduce these parameters in order to ensure biodiesel quality and compliance with the standard. Nevertheless, its high flash point value of 150 °C and its high thermal stability within the temperature range of 211–290 °C suggest the potential for utilization as a biolubricant.

**Keyword** Microalgae · Fatty acid methyl ester · Biofuel · Transesterification

## Introduction

In recent years, society has become increasingly concerned about the depletion of fossil resources. As a result, vegetable-based products have been explored and utilized as substitutes. Microalgae are being researched as a potential alternative to conventional vegetable sources in various industries, including cosmeceuticals, nutraceuticals, and the energy industry. This is due to their ability to produce high-value products, such as carotenoids (astaxanthin, fucoxanthin), lipids, and protein, among others, when grown under stress conditions [1]. The stress conditions during culture and growth are directly influenced by factors such as salinity of the medium, light/dark cycle, type of bioreactor, temperature, dissolved oxygen concentration, agitation, and the contribution of nutrients such as carbon, nitrogen, and phosphorus [2].

Microalgae have several advantages as a raw material, including being a renewable resource, not requiring arable land, and being able to absorb nutrients such as phosphates and nitrates, making them suitable for cultivation in wastewater. They also have a continuous production, high growth rate, and high biomass production and are the largest supplier of O<sub>2</sub> on the planet. Additionally, they are great CO<sub>2</sub> absorbers and have a great diversity of species (> 30,000) [2]. Algal biomass can be used to synthesize various biofuels, including jet fuels, bioethanol, methane, biodiesel, and biobutanol [3]. However, the bio-oil obtained from microalgae is unstable, making its implementation at an industrial scale both difficult and expensive. Additionally, the process requires large quantities of organic solvents for oil extraction from dry biomass, and the harvesting, drying, and extraction of oil from biomass pose significant challenges [4].

*Haematococcus pluvialis* is a unicellular microalga belonging to the volvocalean group. It has a multilayered cellular architecture that includes a central tripartite crystalline layer and a distinct gelatinous extracellular matrix. This microalga is known to be one of the best natural sources of astaxanthin, which can reach up to 5 wt% of dry biomass [5]. Astaxanthin is a natural antioxidant carotenoid synthesized in certain microalgal species. *H. pluvialis* is a source with

✉ E. Rodríguez  
eduardo@uniovi.es

<sup>1</sup> Department of Construction and Manufacturing Engineering, University of Oviedo, Pedro Puig Adam, S/N, 33203 Gijón, Spain

<sup>2</sup> Department of Marine Science and Technology, University of Oviedo, Blasco de Garay, S/N 33203, Gijón, Spain

high astaxanthin content, possessing antiaging, anticancer, anti-inflammatory, and immune-enhancing properties [5].

Triglycerides are molecular structures formed by linking three fatty acids (FAs) through glycerol and can reach a dry biomass content of 80 wt% [6]. These structures comprise the bio-oil and serve as the raw material for synthesizing industrial products, such as biofuels or biolubricants. The FAs of microalgae usually have 16 to 24 carbon atoms, with some containing 5 or 6 double bonds [7]. In contrast, plant crop FAs have shorter chains (8 to 20 carbon atoms) and contain a lower number of double bonds or unsaturations [8]. The molecular structure of biodegradable materials can affect the strength of the C-H bonds, which in turn affects properties such as thermal and oxidative stability. To prevent this, it is advisable to choose a raw material that is free of unsaturation or to eliminate it using complementary techniques such as epoxidation [9].

Figure 1 displays the various stages of biomass production, from cultivation to extraction of the compounds of interest, as well as the various flow lines for the utilization of the residual biomass after astaxanthin extraction. In addition to synthesizing biodiesel through transesterification reaction, other lines of interest may include producing ethanol or methane through processes such as biomethanization (or anaerobic digestion) and hydrolysis [10].

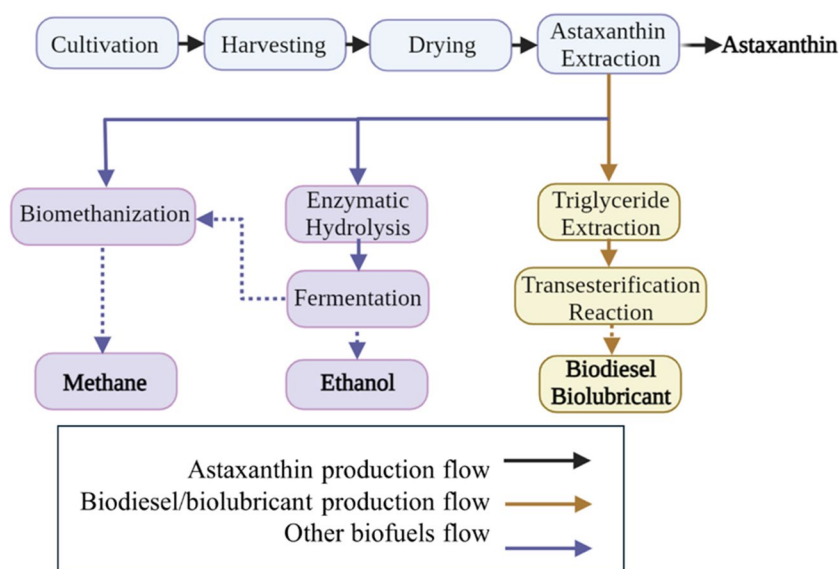
Following the extraction of astaxanthin from *H. pluvialis*, a by-product in the form of biomass residue is generated. This by-product has been identified by other authors as a potential source of value in biorefining routes [5]. The by-product rich in triglycerides, obtained after separating the astaxanthin-containing phase, can be used for biodiesel/biolubricant production. For this purpose, the transesterification reaction is commonly employed, where triglycerides react with an alcohol in the presence of a catalyst and under

specific temperature conditions to yield fatty acid methyl or ethyl esters (FAMES/FAEEs) as the main product and glycerin as a secondary product [11]. The presence of moisture has a significant impact on the reaction, as it reacts with the medium to hydrolyze the fatty acids. The resulting free fatty acids (FFA) react with the alkaline catalyst to form soap [12]. Therefore, it is important to check the moisture and FFA content of the oil prior to the transesterification reaction. When the FFA content is greater than 2–4 mg KOH/g, acid catalysts are recommended [13]. Additionally, the use of cosolvents that homogenize the system and increase the indissolubility of the alcohol with the bio-oil is crucial. Also important is the use of cosolvents that homogenize the system and increase the indissolubility of the alcohol with the bio-oil [12].

Transesterification reaction has been typically optimized using independent variables such as alcohol ratio, reaction time, reaction temperature, catalyst concentration, and stirring speed. This technique has been widely used to optimize biodiesel production and obtain the experimental conditions to reach the highest FAME yield (%) from edible crops (such as sunflower, soybean, and palm), non-edible crops that require arable land (such as *Jatropha* and *Neem*), and microalgae as *Nannochloropsis* sp., *N. gaditana*, or *C. pyrenoidosa* [14–16]. However, the application of the response surface methodology (RSM) to optimize the transesterification reaction of the bio-oil from *H. pluvialis* microalgae has not yet been explored. This microalgae strain has been reported to have great potential as a feedstock for biodiesel/biolubricant production due to its high lipid content (reported as 43%) and rapid growth rate [17, 18].

The aim of this study is to determine the optimal conditions for FAME production from *H. pluvialis* bio-oil by applying RSM to the transesterification reaction and

**Fig. 1** Flowchart for the synthesis of various valuable products from *H. pluvialis* microalgae



determining the physicochemical properties of this modified microalgae bio-oil such as density, viscosity, flash point, pour point, thermal stability, lower and higher heating values, and fatty acid distribution (saturated fatty acids or SFAs, monounsaturated fatty acids or MUFAs, and polyunsaturated fatty acids or PUFAs).

## Material and Methods

### Bio-Oil Characterization

The oil derived from *H. pluvialis* was supplied by Neoalgae Micro Seaweed Products (Gijón, Spain) and was selected following an analysis of its saponifiable lipid (SL) content using the technique described by Callejón [19], wherein the SL content per unit of microalgae bio-oil (MBO) is provided following a transesterification reaction with methanol in the presence of the acid catalyst acetyl chloride. A 50% SL content was determined for the MBO. The lipid profile analysis after the transesterification reaction was conducted following the UNE-EN 14103 standard using a Clarus 690 instrument (PerkinElmer) and gas chromatography with flame ionization detector (GC-FID). The Elite-WAX column (30 m × 0.25 mm × 0.25 µm) was used with hydrogen as the carrier gas flowing at a rate of 2 mL/min. The detector was set at 250 °C, and the oven heating program consisted of the following steps: (1) holding at 60 °C for 2 min, (2) heating from 60 to 200 °C at a rate of 10 °C/min, (3) heating from 200 to 240 °C at a rate of 5 °C/min, and (4) holding at 200 °C for 7 min.

Furthermore, the total acid number (TAN) and water content were quantified using the Metrohm 848 Tritino plus (ASTM D664 standard) and the Metrohm 899 Karl Fischer, respectively, with an estimated precision of ± 0.01 mg KOH/g and ± 0.1 ppm. To determine the best route according to the characteristics of the oil, the

TAN is of great importance. If its value is higher than 2 g KOH/g, it would be necessary to carry out a prior esterification with an acid catalyst.

### Transesterification Reaction

#### Design of Experiments

The transesterification reaction was optimized using a central composite design (CCD) of a two-level factorial approach with RSM and the Design Expert 13 software. Table 1 shows the selected independent variables and parameter levels, which are coded as − 1 (minimum), 0 (center), + 1 (maximum), and ± α (extreme star points). The levels of each parameter were selected according to the bibliography consulted for previous studies, and in addition to this, they were also applied in previous research [20].

Nineteen runs were conducted, consisting of six star points, five center point replications, and eight factorial design runs.

The alcohol used in the reaction was methanol and the catalyst CH<sub>3</sub>ONa was used at a concentration of 1.5% by the weight of oil, in accordance with the literature consulted [21]. A basic catalyst was selected after verifying that the acidity of the oil was less than 2 g KOH/g. Previous tests were also carried out with a basic KOH catalyst, obtaining better results for CH<sub>3</sub>ONa.

To perform the reaction, 1 g of MBO was mixed with 2 mL of hexane, the corresponding amount of methanol (3–15 g) and the catalyst in a 100 mL flask. During the reaction time, the flask was in an oil bath to keep the mixture at the required temperature. A Dimroth condenser was also used to prevent loss of hexane and methanol at higher temperatures.

Finally, the output variable evaluated was the FAME yield (%), calculated according to Eq. 1. The amount of FAME converted was obtained through the analysis described in “Bio-Oil Characterization” using GC-FID and an internal standard of methyl nonadecanoate.

$$Y(\text{FAME yield \%}) = \frac{\text{Amount transformed to FAME (g)}}{\text{Total SL amount convertible to FAME (g)}} \times 100 \quad (1)$$

**Table 1** Input variables and levels for optimizing the MBO transesterification reaction

| Independent variables       | Factor | Variable levels |      |     |       |           |
|-----------------------------|--------|-----------------|------|-----|-------|-----------|
|                             |        | − 2 (− α)       | − 1  | 0   | + 1   | + 2 (+ α) |
| MBO to methanol ratio (wt.) | A      | 1:3             | 1:6  | 1:9 | 1:12  | 1:15      |
| Reaction temperature (°C)   | B      | 30              | 50   | 70  | 90    | 110       |
| Reaction time (min)         | C      | 30              | 67.5 | 105 | 142.5 | 180       |

**Table 2** Design of experiments used in this study

| Standard order | Run order | Factorial input variable |    |    |
|----------------|-----------|--------------------------|----|----|
|                |           | A                        | B  | C  |
| 11             | 1         | 0                        | −2 | 0  |
| 18             | 2         | 0                        | 0  | 0  |
| 2              | 3         | 1                        | −1 | −1 |
| 6              | 4         | 1                        | −1 | 1  |
| 10             | 5         | 2                        | 0  | 0  |
| 4              | 6         | 1                        | 1  | −1 |
| 9              | 7         | −2                       | 0  | 0  |
| 13             | 8         | 0                        | 0  | −2 |
| 19             | 9         | 0                        | 0  | 0  |
| 3              | 10        | −1                       | 1  | −1 |
| 8              | 11        | 1                        | 1  | 1  |
| 1              | 12        | −1                       | −1 | −1 |
| 17             | 13        | 0                        | 0  | 0  |
| 7              | 14        | −1                       | 1  | 1  |
| 14             | 15        | 0                        | 0  | 2  |
| 12             | 16        | 0                        | 2  | 0  |
| 5              | 17        | −1                       | −1 | 1  |
| 16             | 18        | 0                        | 0  | 0  |
| 15             | 19        | 0                        | 0  | 0  |

Table 2 presents the configuration of the test runs using the Design Expert software. Analysis of variance (ANOVA) was performed to determine the interaction of the response  $Y$  with each input variable using Design Expert 13 software. The probability value with a 95% confidence interval was used to evaluate the significance of the model.

### Purification Process

To achieve the purest possible product, a multi-step purification process was followed as described in Fig. 2. Following the transesterification reaction, the product was centrifuged

for 10 min at 7000 rpm (Microcen 24—CE 202) to eliminate glycerol. The resulting liquid was then washed with deionized water to remove excess methanol, traces of catalyst, and other impurities present in the original MBO. This step was repeated until the effluent reached a neutral pH. Finally, transfer the sample to a rotary evaporator set at 60 °C and 20 mbar for 20 min (Hei-Vap Core, Heidolph) to remove any traces of water, methanol, and hexane.

### FAME Physicochemical Characterization

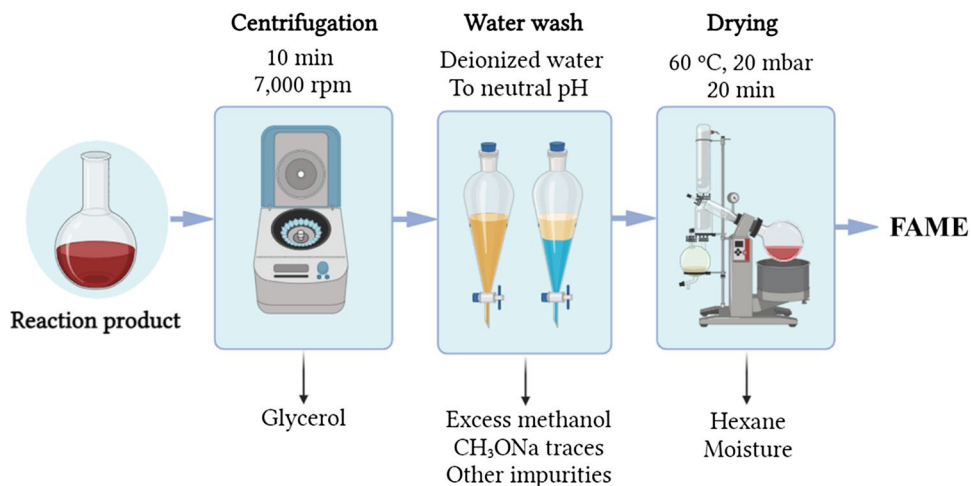
The transformation of the sample from triglyceride to FAME was confirmed through an analysis using a Fourier Transform Infrared (FTIR) spectrometer (Varian 670-IR) with an accuracy of better than  $0.07\text{ cm}^{-1}$ , in addition to GC-FID analysis.

A high-precision Stabinger SVM 3001 rotational viscometer was employed to determine the density at 20 °C and the kinematic viscosity at 40 °C in accordance with the ASTM D7042 standard.

The pour point (PP) was determined through Differential Scanning Calorimetry (DSC Mettler 822e 700) analysis. DSC has a heat flow accuracy of better than  $\pm 2\%$  and a temperature accuracy of  $\pm 1\text{ °C}$ . This technique can directly measure the change in enthalpy for a system during cooling. The change in enthalpy can be estimated using the sample PP [22]. The sample was heated to 50 °C at a rate of 10 °C/min and held under isothermal conditions for 10 min. The system was then cooled to  $-50\text{ °C}$  under a nitrogen atmosphere at the same rate. The PP value can be obtained at the maximum point of the curve by using heat flux (W/g) versus temperature plots [23].

The upper and lower heating values (LHV and HHV) were determined in an IKA C4000 calorimeter according to ISO 1928.

The flash point (FP) of the FAME was determined using a modified Cleveland Open Cup Tester in accordance with

**Fig. 2** FAME purification process


EN ISO 2592 and ASTM D92 standards. The modified cup was filled with 15 mL of FAME and heated in increments of 5 °C until it reached its FP.

The FAME's thermal stability (TS) was then assessed using thermogravimetric analysis (TGA) with a TA instrument that has a temperature accuracy of 0.001 °C (200 to 1300 °C). The results were analyzed using Universal Analysis 2000 software. The sample weighed 6 mg, and it was heated at a constant rate of 20 °C/min from 25 to 600 °C under a nitrogen atmosphere with a flow rate of 50 mL/min. The weight loss of the sample was plotted against time to determine the onset temperature of decomposition.

## Results and Discussion

### Lipid Profile and Molecular Structure

Table 3 presents the results of the lipid profile analysis, which are consistent with data reported for *H. pluvialis* by Bilbao et al. [24] and Damiani et al. [25]. Taking into account the indications of the UNE EN 14214 standard, in which fatty acids with 4 or more double bonds are considered to be PUFAs, *H. pluvialis* presents a PUFA content of 6.02%. The most abundant FAs in the sample are palmitic acid (C16:0) with 20.38%, oleic acid (C18:1) with 15%, linoleic acid (C18:2) with 29.92%, and linolenic acid (C18:3) with 15.21%. All MUFAs and SFAs were identified in the method, indicating that the unidentified peaks belong to PUFAs.

UNE EN 14214: according to the standard, fatty acids must have 4 or more double bonds to be considered a PUFA.

The acidity value of 0.08 mg KOH/g obtained for the MBO indicates the absence of FFA that could interfere with

the reaction. Therefore, an esterification process with an acid catalyst was unnecessary. Additionally, the water content of the sample (1580–1820 ppm) was due to ambient moisture, and thus, a prior drying process was not required.

Figure 3b shows the plot of wave number versus transmittance, displaying the specific molecular bonds present in both MBO and FAME through the infrared spectrum. The wavelength of 1435 cm<sup>-1</sup> represents the -CH<sub>3</sub> bonds, which, together with GC-FID, confirms the transformation taking place in the reaction. The wavelength ranges of 2800–3000 cm<sup>-1</sup>, 1730–1750 cm<sup>-1</sup>, and 1000–1300 cm<sup>-1</sup> correspond to -C-H bonds and -C=O, -C-O esters [26]. In the range of 3000–3700 cm<sup>-1</sup>, the -O-H bonds are present, which strongly relate to the thermal and oxidative stability of the sample [27]. The presence of -O-H molecules increases with the water content, making this wavelength informative since water and moisture contribute to FAME degradation. As shown, this band has a lower presence in FAME compared to MBO, indicating lower water content and better stability of the FAME.

### Optimization of Transesterification Reaction by RSM

Table 4 shows the actual and predicted *Y* response obtained using the Design Expert software and the relative error between the two values.

### Model Adequacy Check

Equation 2 was generated from the empirical model that correlates the coded input variables with the response *Y* through a quadratic regression.

$$Y = 55.77 - 6.94A + 2.70B - 3.9C + 0.8588AB + 2.45AC - 2.73BC + 6.96A^2 + 6.88B^2 + 0.5278C^2 \quad (2)$$

The results of the final ANOVA are presented in Table 5.

Figure 4 shows the interaction between the input variables and the response *Y* (according to Eq. 2) using a 3D surface

**Table 3** Fatty acid composition of *H. pluvialis*

| Fatty acid | Content (%)  | Total                               | (%)          |
|------------|--------------|-------------------------------------|--------------|
| C16:0      | 20.38 ± 0.21 | <b>Not identified</b>               | 8.68 ± 1.04  |
| C17:1      | 2.92 ± 0.02  | <b>SFA</b>                          | 21.23 ± 0.21 |
| C18:0      | 0.85 ± 0.01  | <b>MUFA</b>                         | 18.81 ± 0.12 |
| C18:1      | 15.89 ± 0.13 | <b>PUFA</b> <sup>UNE EN 14214</sup> | 6.02         |
| C18:2      | 29.92 ± 0.07 | <b>Total lipid content</b>          | 50.04        |
| C18:3      | 15.21 ± 0.17 |                                     |              |
| C18:4      | 2.50 ± 0.02  |                                     |              |
| C20:5      | 3.52 ± 0.04  |                                     |              |

plot. It is observed that the reaction time interacts similarly to the alcohol ratio (Fig. 4a) and the reaction temperature (Fig. 4b). *Y* does not respond to time variation, while for the other two variables, it fluctuates significantly. This statement supports the ANOVA results presented in Table 5, where the factors *AC* and *BC* had *p* values greater than 0.05, indicating that they were not significant terms.

In contrast, the relationship between alcohol ratio and temperature indicates a minimum stationary point at [0, 0, 0], with *Y* increasing towards the extremes of [-2, 2]. The highest predicted *Y* values fall within the range of [-1.5, 2] for alcohol ratio and [0, 2] for reaction temperature.

The increase in temperature results in an increase in equilibrium conversion and therefore an increase in FAME yield. Furthermore, it was identified that the effect of temperature

**Fig. 3** **a** DSC at low temperatures, **b** FTIR spectra, and **c** TGA of the MBO and FAME.

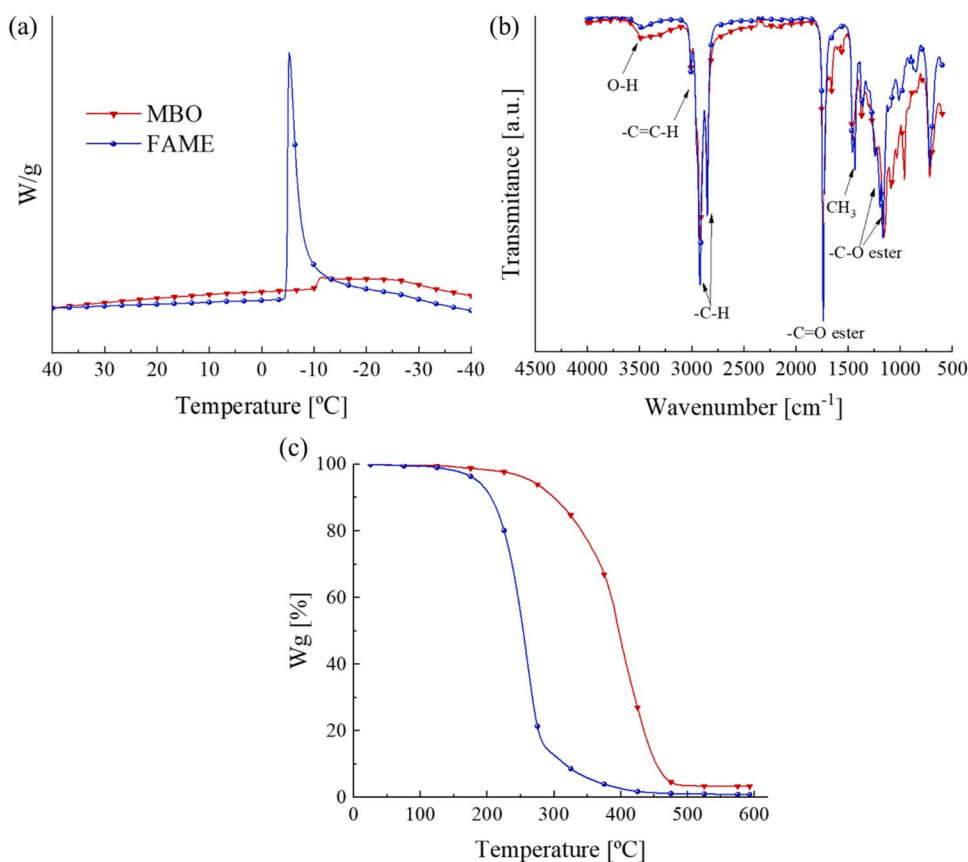


**Table 4** Actual and predicted *Y* response results obtained in this study

| Factorial input variable |          |          | Responses, <i>Y</i> |              |           |
|--------------------------|----------|----------|---------------------|--------------|-----------|
| <i>A</i>                 | <i>B</i> | <i>C</i> | Actual              | Predicted    |           |
|                          |          |          | <i>Y</i> (%)        | <i>Y</i> (%) | Error (%) |
| 0                        | −2       | 0        | 80.48               | 77.89        | 3.21      |
| 0                        | 0        | 0        | 56.89               | 55.77        | 1.96      |
| 1                        | −1       | −1       | 57.65               | 58.37        | 1.25      |
| 1                        | −1       | 1        | 60.94               | 60.91        | 0.04      |
| 2                        | 0        | 0        | 67.84               | 69.73        | 2.78      |
| 1                        | 1        | −1       | 74.01               | 70.93        | 4.16      |
| −2                       | 0        | 0        | 97.23               | 97.49        | 0.27      |
| 0                        | 0        | −2       | 65.23               | 65.69        | 0.71      |
| 0                        | 0        | 0        | 49.12               | 55.77        | 13.55     |
| −1                       | 1        | −1       | 90.12               | 88.00        | 2.36      |
| 1                        | 1        | 1        | 66.12               | 62.57        | 5.37      |
| −1                       | −1       | −1       | 77.47               | 78.87        | 1.81      |
| 0                        | 0        | 0        | 56.93               | 55.77        | 2.03      |
| −1                       | 1        | 1        | 72.71               | 69.84        | 3.95      |
| 0                        | 0        | 2        | 48.39               | 50.08        | 3.48      |
| 0                        | 2        | 0        | 83.94               | 88.68        | 5.64      |
| −1                       | −1       | 1        | 70.69               | 71.62        | 1.31      |
| 0                        | 0        | 0        | 55.36               | 55.77        | 0.75      |
| 0                        | 0        | 0        | 58.42               | 55.77        | 4.53      |

on the FAME yield is less pronounced when reaction times are longer. This allows for high FAME yield at low temperatures to be achieved when utilizing longer reaction times. Kumar also reported this temperature–time balance in a jatropha-algae biodiesel mixture, where the maximum conversion achieved was 96.29% for a time of 180 min and 45 °C [28].

### Optimization Conditions of RSM Analysis

After selecting the empirical model that best fits the experimental dataset and studying the influence of each input variable on *Y*, the optimal conditions for producing FAME from *H. pluvialis* were determined.

The limiting criteria for obtaining optimal conditions were input parameters in range (alcohol ratio, temperature, time) and maximized output (FAME yield) [29].

The optimal conditions obtained in coded format were −1.61 for alcohol ratio, 0.53 for temperature, and −1.54 for reaction time. These values were decoded to obtain the actual experimental configuration, which is tabulated in Table 6. Based on Eq. 2, this configuration gives a predicted *Y* of 103.32%. The experimental tests were conducted under optimal conditions resulting in a FAME yield of 98.44%. This represents a 2.26% error relative to

**Table 5** ANOVA of the fitting model for *Y*

| Source                          | <i>df</i>             | <i>F</i> value               | <i>p</i> value                | Remark                 |
|---------------------------------|-----------------------|------------------------------|-------------------------------|------------------------|
| <i>Model</i>                    | 9                     | 24.95                        | <0.0001                       | Significant            |
| <i>A</i> —MBO to methanol ratio | 1                     | 54.19                        | <0.0001                       |                        |
| <i>B</i> —reaction temperature  | 1                     | 8.17                         | 0.0188                        |                        |
| <i>C</i> —reaction time         | 1                     | 17.15                        | 0.0025                        |                        |
| <i>AB</i>                       | 1                     | 0.4148                       | 0.5355                        |                        |
| <i>AC</i>                       | 1                     | 3.37                         | 0.0995                        |                        |
| <i>BC</i>                       | 1                     | 4.18                         | 0.0712                        |                        |
| <i>A</i> <sup>2</sup>           | 1                     | 80.62                        | <0.0001                       |                        |
| <i>B</i> <sup>2</sup>           | 1                     | 78.75                        | <0.0001                       |                        |
| <i>C</i> <sup>2</sup>           | 1                     | 0.4637                       | 0.5130                        |                        |
| Residual                        | 9                     |                              |                               |                        |
| Lack of fit                     | 5                     | 1.13                         | 0.4664                        | Not significant        |
| <i>Model summary</i>            | <i>R</i> <sup>2</sup> | <i>R</i> <sup>2</sup> (adj.) | <i>R</i> <sup>2</sup> (pred.) | <i>Adeq. precision</i> |
|                                 | 0.9614                | 0.9228                       | 0.7884                        | 17.3295                |

Note that the *F* and *p* values represent the significance of the model. Differences were considered significant at *p* < 0.05. An *F* value of 24.95 and a *p* value < 0.0001 indicate that both the model and its terms are significant. The adjusted *R*<sup>2</sup> of 0.9614 is also consistent with these values. Adequate precision represents the signal-to-noise ratio, so a ratio greater than 4 is desirable, which in this case is 17.3295. The predicted *R*<sup>2</sup>, obtained by comparing the experimental values of *Y* with those obtained by applying Eq. 2, is also in reasonable agreement with the adjusted *R*<sup>2</sup>, with a difference of less than 0.2. The model is appropriate for predicting *Y*, as shown in Table 4, where the actual and predicted values are very close, with a relative error of no more than 6%, except for one of the five replicates of the central point, which presented an error of 13.5% (outlier)

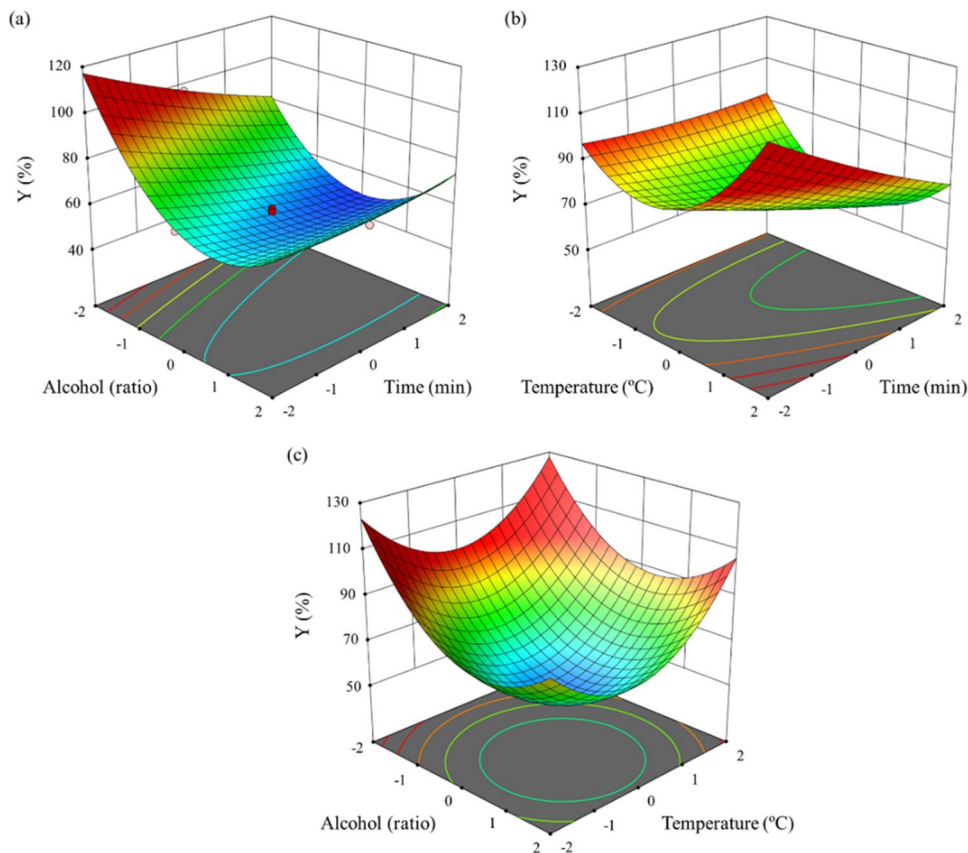
**Fig. 4** Interaction between independent variables for FAME yield. **a** Alcohol ratio vs. reaction time. **b** Temperature vs. reaction time. **c** Alcohol ratio vs. reaction temperature

**Table 6** Coded and real values for optimal transesterification conditions

| Input variable            | Coded value      |               | Real value       |
|---------------------------|------------------|---------------|------------------|
| MBO to methanol ratio     | − 1.61           |               | 4.17             |
| Reaction temperature (°C) | 0.53             |               | 80               |
| Reaction time (min)       | − 1.54           |               | 47               |
| Response, <i>Y</i>        | <b>Predicted</b> | <b>Actual</b> | <b>Error (%)</b> |
| FAME yield (%)            | 103.32           | 98.44         | 2.26             |

the predicted value, which is the highest conversion rate achieved.

### FAME Physicochemical Characterization

The FAME had a density value of 900 kg/m<sup>3</sup>, which is within the range of the UNE EN 14214 standard. This value is significantly higher than that reported for biodiesel from other vegetable sources, such as palm kernel or the microalgae *S. oleosa*, both of which have values of 879 kg/m<sup>3</sup> [30, 31]. However, higher values have been reported, such as for FAME derived from rapeseed oil, with a density value of 916 kg/m<sup>3</sup> [32]. The kinematic viscosity value was slightly elevated, reaching 7.02 mm<sup>2</sup>/s, 2 mm<sup>2</sup>/s above the upper limit specified by standard for biodiesel. This value is also significantly higher than that reported by other vegetable sources, which typically range from 4 to 6 mm<sup>2</sup>/s [33, 34]. Nevertheless, the density and viscosity values of the present study have been determined to fall within the operational range for use as biolubricants, as defined by the SAE J-300 standard. Consequently, it would be of interest to investigate the tribological properties of this biofuel in future studies.

The fluid's working temperature range was determined through a comprehensive thermal analysis. The results of TGA from Fig. 3c shows that FAME has an onset thermal degradation temperature of 211 °C, which is lower than that of the MBO at 348 °C. Additionally, FAME exhibits maximum degradation above 425 °C, resulting in a quasi-complete degradation of the sample. However, the MBO residue remains as it does not fully degrade at the highest test temperature. The onset thermal degradation temperature of FAME derived from *H. pluvialis* has been determined to be higher than that reported for biodiesel derived from jatropha and rapeseed [32, 35]. In comparison with other microalgae, the FAME derived from *S. platensis* and *N. gaditana* strains have been found to exhibit higher degradation onset temperatures, with values of 300 °C and 240 °C, respectively [20, 36]. This observed difference may be attributed to the higher content of unsaturations in the case of *H. pluvialis*, in comparison to the aforementioned last two strains of microalgae, which would have a deleterious effect on its thermal stability.

During the FP measurements, the FAME sample exhibited foaming formation at 100 °C and a continuous ignition from 150 to 155 °C, which is considered the FP temperature. The foaming at 100 °C may be due to the presence of small contaminants, such as other lipids, in the microalgae FAME that break down when heated and tend to form a thin layer of foam in the presence of air [37]. The presence of foam on the surface hinders the dispersion of combustion gases, causing the foam to expand. This FP value is consistent with the typical range observed for FAME derived from vegetal sources, falling between 140 and 190 °C [31, 33].

The determination of PP was challenging for the MBO sample due to its non-pure compound nature, resulting in the absence of a characteristic peak. Instead, it exhibited a broad exothermic freezing band between − 2 and − 32 °C (Fig. 3a). In contrast, the FAME sample displayed a well-defined exothermic freezing peak with an onset temperature of − 4.6 °C and a peak temperature of − 5.1 °C. Additionally, the PP value of the FAME from *H. pluvialis* was slightly lower than that of biodiesel from other vegetable sources. Those derived from edible waste, palm kernel, honge waste cooking oil, or *S. oleosa* have PP values above 0 °C [30, 31, 38]. Lower PP values have been reported for microalgae such as *S. platensis* (− 9 °C) [36]. The reduction in PP in microalgae FAME is attributed to the different molecular characteristics of the fatty acids present in edible vegetable oils and those present in MBOs. In contrast to vegetable oils, MBOs have a higher percentage of polyunsaturated fatty acids, with a value of 6.02% in this study. The presence of cis double bonds in unsaturated compounds hinders crystal packing, resulting in lower PP values [39].

Additionally, the LHV and HHV were determined to be 38.03 and 40.38 MJ/kg, respectively. These values are close to those reported for biodiesel from vegetable sources as jatropha and honge (38.66 and 39.79 MJ/kg, respectively), which in turn are slightly lower than diesel (44.22 MJ/kg) [38].

Most of the important properties exhibited by FAME derived from *H. pluvialis* do not align with the stipulated limits set by the standard. The high viscosity and the PUFA content of 6.02% are significant limitations. It is common for microalgae to contain high levels of PUFAs, with values ranging from 20 to 75% being typical [40]. Although the presence of these structures negatively affects its oxidative stability, the presence of more than one double bond in the carbon chain lowers the viscosity by hindering molecular packing [41]. Consequently, the presence of PUFAs serves to prevent the viscosity from increasing further. Conversely, these values of density, viscosity, and working temperature fall within the operational range for use as a lubricant, in accordance with the SAE J300 Standard for the viscosity grade. Typical lubricant densities range from 700 to 950 kg/m<sup>3</sup>, with operational temperature ranges between 20 and 90 °C for medium load applications [42].

## Conclusions

This research examined the parameters that influence the conversion of bio-oil derived from the microalgae *Haematococcus pluvialis* to fatty acid methyl esters (FAMES) through a transesterification reaction. The optimization process of this reaction was conducted using response surface methodology. An empirical model was obtained, and the optimal conditions for maximizing FAME production were determined. However, the mixture of FAMES from *H. pluvialis* does not meet the standard requirements for classification as biodiesel. Consequently, further research is required to obtain a biodiesel of the requisite quality. Nevertheless, some of the physicochemical properties of this mixture permit its utilization as a biolubricant.

**Author Contribution** C. Sanjurjo: conceptualization, formal analysis, investigation, writing—original draft. E. Rodríguez: investigation, funding acquisition, project administration, writing—review and editing. M. Bartolomé: data curation, formal analysis. R. González: data curation, software. A. Hernández Battez: conceptualization, funding acquisition, investigation, project administration, writing—review and editing.

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**Data Availability** The datasets generated during the current study are available in the ZENODO repository, <https://doi.org/10.5281/zenodo.10689754>.

## Declarations

**Competing Interests** The authors declare no competing interests.

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