Sustainable
Food Technology

ROYAL SOCIETY
OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Sustainable Food Technol.*, 2025, **3**, 1011

Received 24th March 2025
Accepted 8th June 2025

DOI: 10.1039/d5fb00110b

rsc.li/susfoodtech

Sustainable synthesis and optimisation of ethyl oleate from high oleic acid waste: a pathway to valorise industrial byproducts†

Ana Soares,* Ana Luiza Fontes, Francisca Teixeira, Paula Costa, Lígia Pimentel,* Manuela Pintado and Luis Miguel Rodríguez-Alcalá*

Ethyl oleate (EO) is a versatile compound with several industrial applications, such as a vaccine adjuvant, an emollient in cosmetics, and a key component in food products as an additive used for pretreatment in preservation processes such as drying, while preserving valuable nutrients. Ethyl oleate is primarily synthesised from edible oils, which raises concerns regarding competition with food production. This study proposes the use of a high oleic acid waste (HOW) obtained from industrial pipelines as a raw material for EO production, by transesterification with ethanol and using sodium hydroxide as a catalyst. The effects of the HOW : ethanol ratio and recirculated EO addition on both yield and purity levels were investigated. An HOW : ethanol ratio of 6 : 1 (w/w) and a 10% (w/w) of EO recirculated addition resulted in the highest purity (86.16 ± 0.04%) and yield (96.35 ± 0.01%). The resultant EO samples were characterized towards its composition and physicochemical properties. The study highlights the sustainable valorisation of industrial waste. This approach avoids competition with the food chain and offers an eco-friendly method to produce EO for various industrial applications, particularly in food science.

Sustainability spotlight

Conventional ethyl oleate production from edible oils intensifies competition with food resources and contributes to unsustainable practices. Our work introduces an eco-friendly alternative by valorizing high oleic acid waste (HOW), an industrial byproduct, into ethyl oleate *via* an optimized transesterification process. This innovation eliminates reliance on food-grade oils, minimizes waste, reduces energy and reagent use, and diverts byproducts from landfills. By converting waste into a high-value additive for food, cosmetics, and pharmaceuticals, the approach supports circular economy. The process achieves high yield (96.35%) and purity (86.16%) through ethanol reduction and EO recirculation. This aligns with UN SDG 9 (Industry, Innovation, and Infrastructure) by fostering sustainable industrial practices and SDG 12 (Responsible Consumption and Production) through resource-efficient waste valorization.

Introduction

The increasing demand for food products with improved quality and longer shelf-life, alongside the development of more efficient preservation processes, has become major goals of the food industry worldwide in the 21st century.[1] Accordingly, new approaches aiming to improve such procedures' efficiency, while maintaining all product properties (in fruits, vegetables, meat, *etc.*), have been widely studied.[1,2] Regarding the main methods applied in food preservation nowadays, namely, freezing, smoking, vacuum, salting and pickling, sugar, potting, lye, modified atmosphere, and high-pressure conservation, drying is one of the oldest and most commonly used.[3] It consists of reducing the moisture content to a level that allows safe storage of a product for a longer period.[4–6] Thus, removing water from foods is essential to minimize or inhibit microbial growth and deteriorative reactions, while also helping to maintain valuable nutrients such as vitamins. There are several different drying methodologies, including lyophilization (drying by freezing), hot air, a heat pump, microwave drying, cabinet or tray drying, and a fluidized bed or spouted bed, that can be applied in isolation or combined.[5] These conservation processes offer additional advantages, including reduced packaging requirements and lower shipping costs, as the weight and volume of dried products decrease significantly.[7] However, despite the wide range of available drying methods, depending on the characteristics of the product, it may require long operational times, which makes it less sustainable in terms of energy consumption. To overcome this challenge, additives such as preservatives are used to enhance drying kinetics, particularly for small fruits (*e.g.*, blueberries, plums, and mulberries) that have a waxy layer on their peel structure that naturally hinders drying.[4,8–11] Thus, such additives improve the

---

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal. E-mail: amsoares@ucp.pt; lpimentel@ucp.pt; lalcala@ucp.pt

† Electronic supplementary information (ESI) available. See DOI: **https://doi.org/10.1039/d5fb00110b**

drying kinetics, therefore positively impacting the energy cost of the process. They are usually applied as pre-treatments before the drying procedure; (*e.g.*, by dipping the fruit or vegetable into a solution). An example of an emerging additive used for pre-treatment of this type of fruit is ethyl oleate (EO),[4] that also plays an important role in the food industry as a flavouring agent.[12] In commercial applications, EO is primarily synthesized from oleic acid-rich sources such as vegetable oils.[13] However, this practice competes with food production, negatively impacting the food chain. Accordingly, it would be useful to find alternative sources of raw materials with high oleic acid content.

Regarding their synthesis, fatty acid ethyl esters, such as EO, can be synthesized through two processes: the first one occurs directly through esterification of fatty acids with ethanol and catalysed by acids.[14–17] The second one, more common in the industrial field, takes advantage of the presence of triglycerides in oils using a transesterification with ethanol but in the presence of alkaline catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH) or the respective alkoxides.[18–20] In both processes, heating, excess ethanol, and a catalyst are mandatory to increase reaction kinetics and yield.[19–21] In the industrial field, due to resource availability, the most frequently applied strategy is the transesterification of triglycerides (from vegetable oils) catalysed by NaOH, due to the low cost of the catalyst and high yields achieved.[22] Recently, there has been growing focus on utilizing ingredients rich in triglycerides derived from industrial waste within the circular economy framework.[23] These ingredients are obtained through more sustainable processes, and their potential in the food industry has garnered significant attention. It has been estimated that food loss and waste (FLW) has a significant impact on global green-house gas (GHG) emission of 6.8 Gt-CO_2 eq per year;[24] thus, a 50% reduction of FLW could result in a 21% decrease in the associated GHG. In this context the reutilization of vegetable oils can make a significant contribution. According to the FAO[25] from 1.6 billion hectares (ha) used in cropland, 343 million ha are dedicated to oil crops and the carbon footprint of vegetable oils has been calculated to be up to 2.47 kg CO_2 eq per kg rapeseed, 2.94 kg CO_2 eq per kg sunflower, 3.73 kg CO_2 eq per kg palm and 4.24 kg CO_2 eq per kg soybean.[26] This is put into context if the production for those oils is considered: 74 million tonnes of palm oil, 60 million tonnes for soybean, 26 million tonnes of rapeseed and 20 million tonnes of sunflower.[25] Therefore, the development of processes for their reutilization provides an excellent opportunity to reduce their environmental impact.

Consequently, various approaches have been undertaken to develop ingredients that can serve as additives in food, aiming to enhance food preservation, improve quality, increase shelf-life, and ensure consumer safety.[27] During in-depth investigation of lipid profiles in fermentation by-products, the authors discovered that a previously underexplored waste material showed promising potential as a viable raw material for EO production through base-catalyzed transesterification. This research study focused on the valorisation of an industrial waste, known as High Oleic Waste (HOW), which is rich in triglycerides containing oleic acid. The objective was to synthesize ethyl oleate by transesterification with ethanol. The optimisation of the synthesis process aimed to minimize the required amount of ethanol and enhance the purity and yield of the final product by recirculating ethyl oleate within the system. Throughout the study, temperature, reaction time, and catalyst amount were kept constant.

Experimental

Materials

High Oleic Waste (HOW) was kindly provided by Amyris, Inc. (Emeryville, CA, USA). Ethanol (96% (v/v), food grade), ethyl acetate (99% (v/v), food grade), *n*-hexane (HPLC Grade, 97%), dimethylformamide (≥99.9%, HiPerSolv CHROMANORM® for HPLC), methanol (≥99.9%, HiPerSolv CHROMANORM® for HPLC-MS)), sulfuric acid (ACS reagent, 95–98%), sodium hydroxide pellets (NaOH, ACS reagent, 98%), citric acid (ACS reagent, 99%) and sodium chloride (NaCl, ACS reagent, 99%) were purchased from VWR Chemicals (Radnor, Pennsylvania, USA). Methanol (HPLC grade, 99.9%), acetonitrile (HPLC grade, 99.9%), tetrahydrofuran (suitable for HPLC, ≥99.9%), acetic acid (for HPLC LiChropur, 100%), glyceryl tritridecanoate (≥99.0%), the ethyl oleate standard (98%) and the Supelco 37 FAME mixture standard (CRM47885) were purchased from Sigma Aldrich (Merck KGaA, Darmstadt). Ultra-pure water (upH2O) was obtained through a Milli-Q system (Merck Millipore, Burlington, MA, USA) coupled with a 0.22 μm membrane filter (Millipak; Merck Millipore).

Methods

Fatty acid methyl ester (FAME) preparation. For the determination of the fatty acid (FA) composition of HOW, it was weighed (3 mg) and derivatized according to Pimentel *et al.*,[28] with some modifications. Briefly, the samples were mixed with 200 μL of glyceryl tritridecanoate. Then 2.26 mL of methanol were added, followed by 1 mL of *n*-hexane and 240 μL of sodium methoxide (5 M). The samples were homogenized and incubated at 80 °C for 10 min. After cooling in ice, 1.25 mL of DMF were added prior to 1.25 mL of methanolic sulfuric acid (3 M; prepared daily). The samples were homogenized and incubated at 60 °C for 30 min. Finally, after cooling in ice, 1 mL of *n*-hexane was added, and the samples were vortexed and centrifuged (1250*g*; 18 °C; 5 min). After derivatization, the organic phase containing fatty acid methyl esters (FAMEs) was collected for further analysis by GC-FID.

Lipid profiling by high performance liquid chromatography–evaporative light scattering detector (HPLC–ELSD). The samples were accurately weighed and dissolved in dichloromethane at a concentration of 3 mg mL^{-1}. Then, the samples were analyzed on an HPLC (model 1260 InfinityII, Agilent Technologies, Santa Clara, CA, USA) attached to an Evaporative Light Scattering Detector (ELSD, 1290 Infinity II, Agilent Technologies, Santa Clara, CA, USA) using nitrogen as the nebulizing gas coupled to a Zorbax RX-SIL column (2.1 × 150 mm, 5 μm, Agilent Technologies, Santa Clara, CA, USA). The analysis conditions were assayed, as described by Abreu *et al.*[29] with slight modifications as described by Teixeira *et al.*.[30] The compositions of the mobile phases were as follows: (A) isooctane/ethyl acetate (99.8 : 0.2, v/v); (B) acetone/ethyl acetate (2 : 1, v/v) containing 0.1% acetic acid (v/v); (C) 2-propanol/water (85 : 15, v/v) containing 0.013% acetic acid (v/v) and 0.031% of TEA v/v; (D) EtAc. The flow rate was set at 0.275 mL min^{-1} and an injection volume of 20 μL was used.

The detector was set as follows: evaporator and nebulizer temperatures were set at 60 °C with nitrogen as the nebulizing gas at 1.20 SLM flow rate. To determine the elution order, pure standards were injected and available bibliography was used.[31] In all analyses performed, all the samples were injected at least in triplicate.

Experimental design for the optimisation of ethyl oleate synthesis from HOW. The optimisation process of the synthesis of ethyl oleate (EO) from HOW, by transesterification of triglycerides present in the residue considered variables such as the ethanol : HOW ratio (w/w) and the introduction of ethyl oleate into the reaction system (recirculated) (Table 1). The design of this optimisation study is based on the previous studies of Ortega *et al.* (2021) and Anastopoulos *et al.* (2009) in the field of the transesterification of vegetable oils and waste oils with ethanol to produce biodiesel.[20,21] These authors studied the effect of parameters such the ethanol ratio, amount of catalyst, temperature, and reaction time on the production of fatty acid ethyl esters (FAEEs) from waste frying oils and vegetable oils, respectively. The best reported conditions of transesterification were (a) a ratio of ethanol of 6 : 1, 1% (w/w) of catalyst at 60 °C and 3 h of reaction time, and (b) a ratio of ethanol of 12 : 1 (w/w), 1% of NaOH (w/w) at 80 °C and 2.5 h of reaction time, respectively. Nevertheless, the use of vegetable oils to produce FAEEs is not desirable due to the competition with food production. This research work aims for the use of an industrial waste rich in oleic acid, as a raw material for the production of ethyl oleate without impact on food production processes. The effect of the ethanol ratio will also be evaluated, as well as the introduction of recirculated ethyl oleate in the synthesis process to shift the reaction towards EO production.

The temperature and reaction time were kept constant during this study at 70 °C and 3 h and the different ethanol ratios and introduction of EO recirculated conditions are presented in Table 1.

The first variable studied was the effect of the ethanol ratio on the transesterification reaction to produce EO. Three ratios were evaluated: 12 : 1 (w/w), 9 : 1 (w/w) and 6 : 1 (w/w). The amount of catalyst was maintained at 1% (w/w) of NaOH (ethanolic solution, 2.5 M). For each experimental condition, three independent reaction batches were prepared and processed separately.

Table 1 Reaction conditions tested for synthesis of ethyl oleate from transesterification of HOW with ethanol

Sample	HOW (g)	EO (g)[a]	EtOH : HOW ratio (w/w)
EO12 : 1	5	n.a.	12 : 1
EO9 : 1	5	n.a.	9 : 1
EO6 : 1	5	n.a.	6 : 1
EO9 : 1R_10 g	9	1	9 : 1
EO6 : 1R_10 g	9	1	6 : 1

[a] Ethyl oleate from the previous EO9 : 1 batch, n.a. (not applicable).

The second variable studied was the addition of 10% (w/w) of EO in the initial mixture for the transesterification reaction. Adding EO at the beginning of the reaction was intended to shift the equilibrium toward EO production. This addition was tested using two different ratios of ethanol, 9 : 1 (w/w) and 6 : 1 (w/w) (Table 1).

General optimized synthesis procedure from HOW. The optimised procedure (EO6 : 1R) was carried out with 9 g of High Oleic Waste (HOW) and 1 g of ethyl oleate (previously synthesised) in a round bottom flask where ethanol was added (69.3 mL; ethanol : HOW ratio - 6 : 1, w/w) with ethanolic solution of NaOH 1% (w/w) (4.5 mL, 2.5 M). The mixture was heated with stirring at 70 °C for 3 h. Afterwards, the ethanol was evaporated using a rotary evaporator Heidolph HeiVAP (Schwalbach, Germany) and recovered (≅97%). The crude mixture was neutralized (*i.e.* pH 7.0) with 1% of citric acid solution, producing a mixture of FAEEs. After neutralization, FAEEs were extracted using a separation funnel with ethyl acetate (3 × 30 mL) and 3% NaCl aqueous solution (30 mL). The organic phase was evaporated in the rotary evaporator where it was possible to recover ethyl acetate (≅97%). The FAEE mixture was dried in a ThermoFisher Scientific Oven (Waltham, Massachusetts, USA) overnight at 50 °C and obtained as a pale orange oil.

As a final test, a final batch of EO was prepared using 230 g of HOW under the conditions described in this section.

Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) measurements. The acquisition of FTIR-ATR spectra was performed for all samples of EO synthesised, EO Sigma (commercial) and HOW, on a PerkinElmer Paragon 1000 FTIR (Waltham, Massachusetts, United States) with an ATR accessory, incorporated with a Diamond/ZnSe crystal. The FTIR-ATR spectra were measured in transmittance mode with 16 scans and 4 cm^{-1} resolution in the wavenumber range of 4000–550 cm^{-1} according to conditions previously reported by the authors.[32] The data were processed using the software Spectrum 10.1.0. The FTIR- ATR vibrational bands were identified based on the literature.[33]

The conversion rate of transesterification was calculated by using the ratio between the area of the following bands: 1036 cm^{-1} and 1465 cm^{-1} from the absorbance spectra as previously described by Ortega *et al.*[21]

Differential scanning calorimetry (DSC) measurements. Differential scanning calorimetry measurements were performed using a NETZSCH DSC 204 F1 Phoenix (NETZSCH-Gerätebau GmbH, Selb, Germany) calorimeter as described by Teixeira *et al.*[30] with slight modifications. The samples (2–3 mg) were prepared in duplicate by weighing them in aluminium crucibles and sealing them. Runs were performed as a cycle of various alternating heating and cooling steps to determine the melting, crystallisation and degradation temperatures of the samples. Step 1: heating from 20 °C to 110 °C, step 2: cooling from 110 °C to −60 °C, step 3: heating from −60 °C to 250 °C, step 4: cooling from 250 °C to −60 °C and step 5: heating from −60 °C to 500 °C. Each step was performed at a rate of 10 °C min^{-1} including an isothermal step of 1 min at the end of each heating/cooling step. A nitrogen flow rate of 100 mL min^{-1} was maintained during the runs and an empty and sealed crucible was used as the reference. The discussed data were extracted from the steps 3 to 5. Steps 1 and 2 were performed to eliminate possible moisture content.

Gas chromatography with flame-ionization detection (GC-FID) analysis. The following GC-FID conditions are suitable for the analyses of both FAMEs and FAEEs. Thus, previously prepared FAMEs extracted from the HOW sample or FAEEs (*i.e.* EO samples) dissolved in *n*-hexane (0.5 mg mL^{-1}) were further analysed in a GC (7890B; Agilent Technologies, Santa Clara, CA, USA GLC-FID) apparatus with a FID (Flame Ionization Detector) (GLC-FID) equipped with a BPX70 column (60 m × 0.25 mm × 0.25 μm, SGE Trajan), using the method described by Fontes *et al.*[34] For the analysis, the injector was set at 250 °C with a 25 : 1 split, and the detector temperature at 275 °C. The sample (1 μL) was injected using hydrogen as the carrier gas at 20.5 psi, and the oven temperature was initially 60 °C (held 5 min), and then increased at 15 °C min^{-1} to 165 °C (held for 1 min) and finally at 2 °C min^{-1} to 225 °C (held for 2 min). The identification of FAMEs was carried out through comparison of their chromatographic profile with that of a Supelco 37 FAME mixture standard (CRM47885). Identification of EO was performed by comparing retention time after injecting a standard (*i.e.* Sigma) *versus* the samples (ESI Fig. S1†).

Statistical analysis. The results are reported as mean values ± standard deviation. Data were first analysed for the normality distribution (*i.e.*, Shapiro–Wilk). Levene's test was applied to verify the homogeneity of the variances. Afterwards, a one-way ANOVA test was applied with the Bonferroni post hoc test to determine differences within groups. The level of significance was set in general at 0.05. Analyses were performed with the aid of the IBM SPSS Statistics software (28.0 version, Chicago, USA).

Results and discussion

Fatty acid (FA) composition and triglycerides content in HOW

In order to validate the use of High Oleic Waste (HOW) for the synthesis of ethyl oleate using a transesterification procedure, it is relevant to determine its content in oleic acid. Hence, the fatty acid composition of HOW was evaluated by GC-FID (Table 2).

Analysis results showed that HOW contains a high percentage of oleic acid (84.767 ± 0.006%), the predominant fatty acid in its composition. Other relevant compounds identified were linoleic acid (5.574 ± 0.001%) and palmitic acid (3.51 ± 0.006%). HPLC–ELSD analysis further revealed that HOW has a triglyceride (TG) content of 99.5 ± 0.01% (Table 3), consistent with a vegetable oil nature.

Given the high concentration of oleic acid and TGs in HOW, it is a suitable candidate for use as a precursor in the synthesis of ethyl oleate through transesterification with ethanol in the presence of an alkaline catalyst such as NaOH. This process can effectively convert the fatty acids in HOW into FAEEs, such as ethyl oleate, which has various industrial applications. The proposed reaction of transesterification is schematized in Fig. 1.

Table 2 Fatty acid composition (%) of high oleic waste by GC-FID[a]

	Relative abundance	
Fatty acid	Mean	SD
Palmitic acid (C16)	3.51	0.006
Palmitoleic acid (C16 : 1)	0.189	0.007
Stearic acid (C18)	3.03	0.01
C18 : 1 (all trans)	0.21	0.03
Oleic acid (C18 : 1 c9)	84.767	0.006
Vaccenic acid (C18 : 1 c11)	0.768	0.009
Linoleic acid (C18 : 2 c9 c12)	5.574	0.001
α-Linolenic acid (C18 : 3 c9 c12 c15)	0.077	0.006
Arachidic acid (C20)	0.305	0.005
Gondoic acid (C20 : 1 c11)	0.3011	0.0006
Behenic acid (C22)	0.952	0.007
Lignoceric acid (C24)	0.32	0.02
Σ SFA	8.11	0.03
Σ MUFA	86.24	0.03
Σ PUFA	5.651	0.007

[a] Average values ± standard deviation ($n = 3$). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 3 Lipid composition (%) of HOW[a]

	Relative abundance	
Fatty acid	Mean	SD
Wax esters	0.0146	0.0008
Triglycerides	99.5	0.01
Fatty alcohols	0.02	0.01
Phytosterols	0.01	0.001
Diglycerides	0.302	0.008
Free fatty acids	0.157	0.001

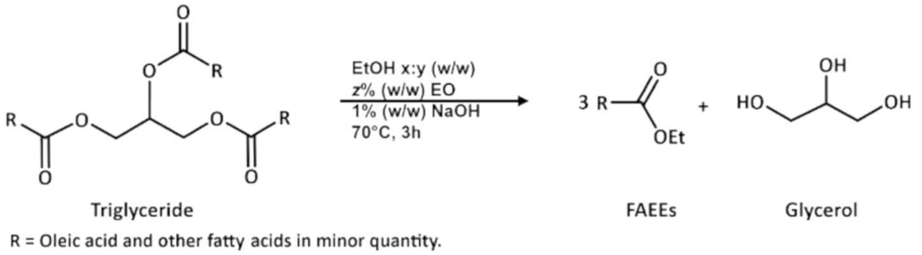
[a] Average values and standard deviation ($n = 3$).

The transesterification reaction should take place in all triglycerides (TGs) present in the feedstock, such as high oleic oil (HOW). In this reaction, the R group, which represents a fatty acid (such as oleic acid or other fatty acids present in minor quantities), reacts with an excess of ethanol in the presence of a catalytic amount of sodium hydroxide (1% w/w). The reaction is typically carried out at a temperature of 70 °C for a duration of 3 hours.

To further enhance yield and purity of the resulting EO, the introduction of recirculated EO was also evaluated. This approach aimed to increase overall transesterification efficiency and improve the final product quality.

Structural characterization of EO samples and the conversion rate of transesterification by fourier transform infrared spectroscopy-attenuated total reflection (FTIR-ATR)

The FTIR-ATR analysis was performed on HOW, EO Sigma (commercial reference) and on all the EO samples obtained during the optimisation process (Fig. 2).



Where x is the amount of EtOH, y is the amount of HOW and z is the amount of ethyl oleate (EO).

Fig. 1 Schematic representation of the synthesis of FAEEs, namely ethyl oleate by the transesterification reaction of a triglyceride with ethanol.

The HOW spectrum (black line) exhibits a distinctive FTIR-ATR vibrational band at 1745 cm^{-1}, corresponding to the C=O stretching of the ester bond found in triglycerides. This observation is consistent with previous findings obtained through HPLC-ELSD analyses. In contrast, the FTIR-ATR spectrum of EO Sigma (dashed line) displays a distinct vibrational band at 1737 cm^{-1}, attributed to the C=O stretching of FAEEs (fatty acid ethyl esters). Notably, all the EO sample spectra (grey lines), resulting from the transesterification of HOW, exhibit the latter vibrational band, indicating the successful completion of the synthesis procedure. These results are further supported by Cataldo,[35] while studying the conversion of ethyl oleate into secondary ozonides and their thermal degradation and photolysis, highlighting the appearance of a band associated with the C=O stretching of ethyl oleate at 1738 cm^{-1}. Also in that work, the reported FTIR spectra showed bands between 3000 cm^{-1} and 2700 cm^{-1} that were also observed in the current research work, specifically in our case at 3010 cm^{-1}, 2923 cm^{-1} and 2853 cm^{-1}. It has been explained elsewhere that the first band corresponds to the C–H axial deformation of the double bond while the other two result from the anti and symmetric stretching vibrations of both C–H in methyle (*i.e.* CH_2) and methyl (*i.e.* CH_3) groups.[36]

Triglyceride conversion into FAEEs, through determination of the transformation rate, was assessed using the method described by Ortega *et al.*[21]. The rate is derived from the ratio between areas under the vibrational bands at 1036 cm^{-1} and 1465 cm^{-1}, as illustrated in Fig. 3. This approach allowed us to monitor conversion progress, providing valuable insights into triglyceride transformation into FAEEs.

The first band is related to the deformation vibration of the C–O bond in the $–OCH_2–CH_3$ of ethyl groups of FAEEs and the second band is related to the deformation vibration frequency of CH_2 groups of triglycerides. The FTIR spectra of HOW and EO samples were converted from transmittance to absorbance, and the above-mentioned areas were assessed (function provided by the software). The obtained conversion rate values are summarized in Table 4.

The conversion rates were calculated to determine whether the optimization variables—namely, the ethanol-to-HOW ratio and the addition of 10% recirculated EO—affected this parameter. As shown in Table 4, the conversion rates for the three ethanol ratios were similar *($p > 0.05$)*, with values of 0.68 ± 0.02 for EO12 : 1, 0.70 ± 0.02 for EO9 : 1, and 0.68 ± 0.02 for EO6 : 1. This indicates that even a lower ethanol amount can achieve efficient transesterification. In pursuit of a more cost-effective and environmentally sustainable process by reducing ethanol usage, the 12 : 1 ratio was not tested in subsequent assays. Notably, a similar outcome was observed in the work of Anastopoulos *et al.*,[20] where a single-stage transesterification of

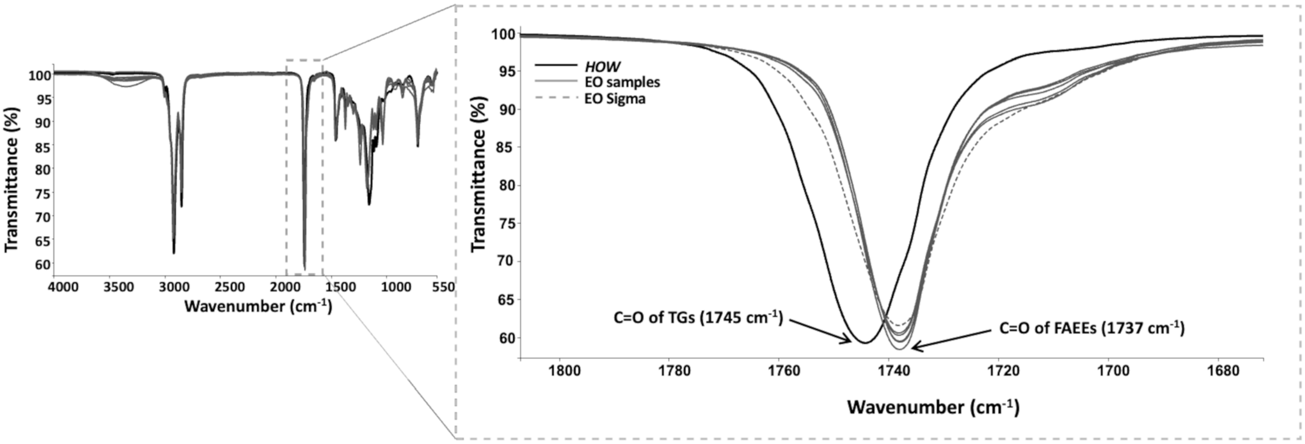


Fig. 2 FTIR-ATR spectra represented in absorbance of all EO samples synthesized, EO Sigma (commercial) and High Oleic Waste (HOW).

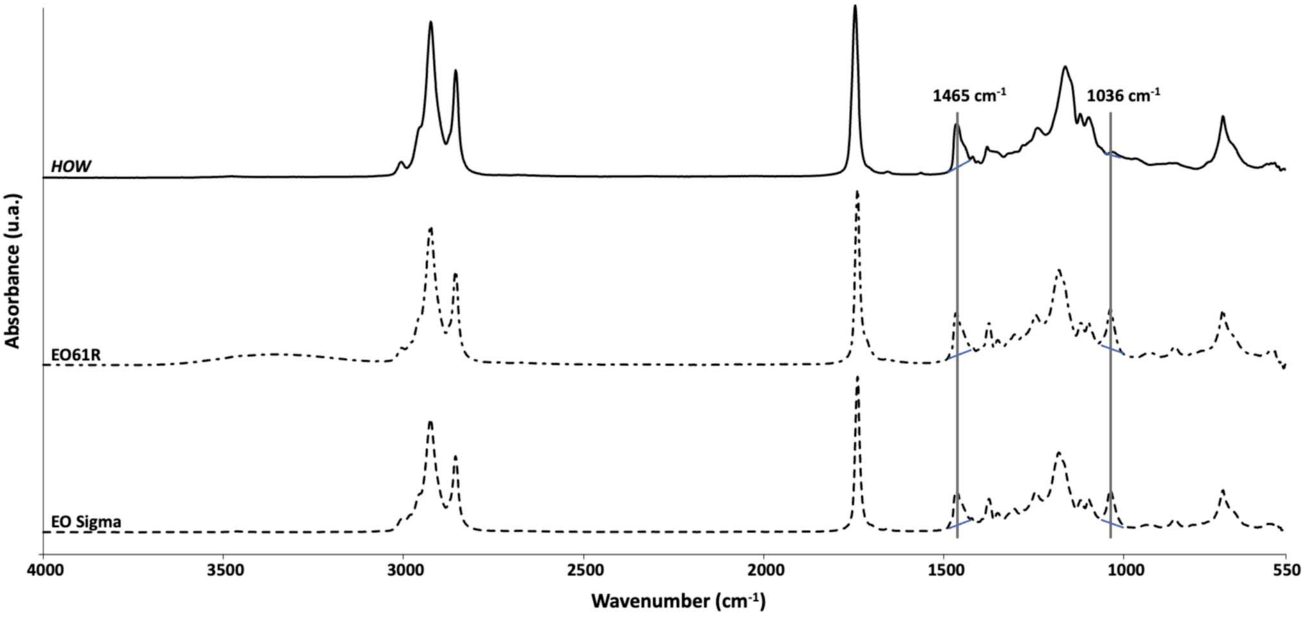


Fig. 3 FTIR-ATR spectra represented the absorbance of all EO samples synthesized, EO Sigma (commercial) and High Oleic Waste (HOW).

Table 4 FTIR spectra monitoring triglyceride conversion to fatty acid ethyl esters (FAEEs)[a]

Sample	Absorbance ratio 1036 cm^{-1}/1465 cm^{-1}	
HOW	0.04	±0.01^{c}
EO12 : 1	0.68	±0.02^{b}
EO9 : 1	0.7	±0.02^{b}
EO6 : 1	0.68	±0.03^{b}
EO9 : 1R	0.73	±0.01^{b}
EO6 : 1R	0.87	±0.01^{a}

[a] Average values ± standard deviation ($n = 3$). Different superscript letters for statistically significant differences ($p < 0.05$) within rows.

sunflower oil using a 12 : 1 ethanol-to-oil ratio, 1 wt% NaOH, 80 °C, and 3.5 hours resulted in an 81.4% yield; a second stage was then required to increase the overall yield to 96.5%.

Including ethyl oleate in the transesterification reaction enhances conversion by shifting equilibrium toward product formation (*i.e.* Le Chatelier's principle). Additionally, ethyl oleate acts as a co-solvent, improving ethanol-oil miscibility, enhancing mass transfer, and reducing reaction mixture viscosity. These effects, combined with potential reduction in side-reactions like saponification, collectively contribute to more efficient conversion.

Accordingly, this effect was evaluated by comparing EO9 : 1 with EO9 : 1R and EO6 : 1 with EO6 : 1R. The conversion rates for EO9 : 1 and EO9 : 1R were 0.7 ± 0.02 and 0.73 ± 0.01, respectively, showing no significant difference. However, a marked increase was observed for EO6 : 1R (0.87 ± 0.01) compared to EO6 : 1 (0.68 ± 0.04), suggesting a synergistic effect between the ethanol ratio and recirculated EO.

Overall, these findings demonstrate that incorporating recirculated EO—especially with a low ethanol amount—can significantly enhance the transesterification reaction.

Reaction yield and purity

Table 5 presents the results regarding the reaction yield (w/w) and EO purity (%) of the obtained EO samples when assaying different reaction conditions.

To evaluate how the ethanol-to-residue (w/w) ratio impacts reaction yield and EO purity, three different ratios (12 : 1, 9 : 1, and 6 : 1) were tested. Using an excess of alcohol is a common practice in transesterification because the reaction is reversible,

Table 5 General yields in percentage of weight and EO purity by GC-FID (FAEE relative abundance).[a]

	% yield (w/w)		
Experiment	Mean	SEM	%RSD
EO12 : 1	85.6	0.9^{d}	1.9
EO9 : 1	88.6	1.2^{c,d}	2
EO6 : 1	89.1	0.3^{c}	0.6
EO9 : 1R	96.59	0.006^{b}	0.01
EO6 : 1R	96.35	0.006^{b}	0.01
EO purity (%)			
EO12 : 1	85.93	0.02	0.05
EO9 : 1	85.92	0.05	0.09
EO6 : 1	85.8	0.09	0.17
EO9 : 1R	86.2	0.006	0.012
EO6 : 1R	86.16	0.02	0.05

[a] Data as average values ($n = 3$), standard error of the mean (SEM) and Relative Standard Deviation (%RSD). Different superscript letters for statistically significant differences ($p < 0.05$) within rows.

and additional alcohol helps drive the equilibrium toward product formation. As shown in Table 5, reducing the ethanol ratio from 12 : 1 to 9 : 1 increased the reaction yield from 85.6 ± 0.9% (EO12 : 1) to 88.64 ± 1.2% (EO9 : 1), while EO purity, determined by GC-FID, remained similar (85.93 ± 0.02% *vs.* 85.92 ± 0.05%). Further lowering the ratio to 6 : 1 resulted in an even higher yield (89.1 ± 0.3%), with no notable change in purity (85.8 ± 0.09%).

The obtained results (85.6–89.1%) were higher than those reported by Anastopoulos *et al.*,[20] who achieved yields of 81.4%. It is important to note that their study was conducted using frying oil rich in linoleic acid (*i.e.*, sunflower oil), whereas our study used HOW. These differences in performance are likely due to the compositional variations between the oils; specifically, the sunflower oil used by Anastopoulos *et al.* had a free fatty acid (FFA) content of 8.3%, while the oil in our study contained lower amounts (0.157 ± 0.001%; Table 3). Elevated FFA levels (above 2–3%) combined with an alkali catalyst can lead to soap formation, thereby reducing process effectiveness.[37] Some researchers have proposed a two-step process that first employs an acid catalyst, such as sulfuric acid—which exhibits slower kinetics[38] —and then an alkali catalyst, achieving yields of up to 97.3%.[39]

Building on these findings, the next step in the optimization of the process involved adding 10% (w/w) recirculated EO at the start of the reaction for the 9 : 1 and 6 : 1 ratios. This approach aimed to shift the equilibrium further toward ethyl ester production, thus increasing the yield of the process.

The results in Table 5 show a slight but not statistically significant increase in EO purity when recirculated EO was added, from 85.92 ± 0.05% (EO9 : 1) and 85.8 ± 0.09% (EO6 : 1) to 86.2 ± 0.006% (EO9 : 1R) and 86.16 ± 0.02% (EO6 : 1R). More notably, the reaction yield increased substantially to 96.59 ± 0.006% (EO9 : 1R) and 96.35 ± 0.006% (EO6 : 1R), compared to 88.6 ± 1.2% (EO9 : 1) and 89.1 ± 0.3% (EO6 : 1) without addition of EO.

The use of lipases such as porcine lipase[40] or *Rhizopus* sp. lipase[41] through fermentation has been studied elsewhere, carrying out the reaction between oleic acid and ethanol in the presence of an organic solvent, and hexane has been reported to be the most suitable. In the case of the assays using *Rhizopus* sp., it was found than 97.8% of the substrate was transformed into EO in 1 hour at 45 °C. However, this study did not explore the utilization of EO synthesis from oil, as in this research, which would increase its applicability.

Other authors such as Zheng *et al.*[42] reported that a Brønsted acidic-surfactant-combined ionic liquid (*i.e.* 3-(*N*,*N*-dimethyldodecylammonium propanesulfonic acid hydrogen sulfate) was used to perform the esterification of free oleic acid with ethanol in water. Under the assayed conditions (5 wt% of catalyst, 3 : 1 ethanol to oleic mol ratio, 0.1% water, 3 h at 78 °C), the reaction yield was 94.6%, while using 0.4% of water increased the value up to 97.1%. As with other previously mentioned investigations, this work did not provide information of the suitability of using oils as a source of oleic acid which would give an idea of suitability for industrial applications.

The results presented in this manuscript highlight that introducing recirculated EO can significantly enhance the transesterification process, increasing both yield and product purity by favourably shifting the reaction equilibrium toward fatty acid ethyl esters (FAEEs). This approach when compared with those previously discussed, allows us to perform the synthesis in one single step, with the subsequent saving of energy, reagents and time.

Differential scanning calorimetry (DSC) analysis

Differential scanning calorimetry analysis was performed in order to characterize the EO samples towards its melting, crystallisation and degradation temperatures. The results are presented in Table 6.

Regarding the crystallisation temperatures, the synthesised samples presented higher temperatures (ranging from −44.9 ± 0.8 °C to −42.1 ± 0.3 °C) than the EO Sigma (−50.00 ± 0.01 °C). On the other hand, the melting temperatures of the EO samples were lower (varying from −22.85 ± 0.15 °C to −30.00 ± 0.01 °C) than the one measured for the EO Sigma (−14.70 ± 0.01 °C). Concerning degradation, the temperatures measured for the EO samples were lower (from 345.0 ± 0.2 °C to 353.7 ± 1.1 °C) than the one associated with EO Sigma (383.4 ± 0.01 °C). These slight variations in crystallisation, melting and degradation temperatures may be related to the presence of other FAEEs in the obtained mixture, once it is known that HOW is rich in triglycerides (Table 3) and oleic acid (Table 2) but also other fatty acids, such as linoleic, palmitic, stearic, behenic and vaccenic acid although to a much lesser extent. For the samples EO12 : 1, EO9 : 1 and EO6 : 1 a second transition consistent with crystallisation was observed between −52.7 ± 1.9 °C and −51.40 ± 0.01 °C, but it was not detected in the other samples, when the recirculated EO was added. This could be associated with the higher purity levels of the latter samples. Relative to HOW, characteristic thermal transitions were also observed namely crystallization at −47.45 ± 0.15 °C, melting at −8.6 ± 0.3 °C and degradation at 423 ± 16 °C, and these transitions are distinct from those of the EO samples.

Exploring the suitability for scaling-up the proposed methodology

Initial laboratory-scale experiments for EO production were conducted using 9 g of HOW. To evaluate the feasibility of larger-scale production, a subsequent experiment was performed by processing 230 g of HOW, the maximum quantity manageable with our laboratory setup, to produce EO. This represented a 25-fold increase in process scale.

The results from this scaled-up experiment (Table 7) indicated no significant differences in EO yield, purity, or thermal properties compared to those of the smaller-scale trials (*i.e.* 9 g of HOW). The developed procedure is designed for straightforward implementation in standard research facilities, utilizing readily available reagents (preferably of food-grade purity where applicable) and without requiring sophisticated equipment.

Table 6 Temperature transitions obtained by DSC for HOW, EO samples (synthetised) and EO Sigma (commercial).[a]

Sample	Crystallisation (°C)				Melting (°C)		Degradation (°C)	
HOW	−47.45 ± 0.15^{a}				−8.6	±0.3^{e}	423	±16^{a}
EO12 : 1	−52.7	±1.9	−44.4	±0.7^{bc}	−26	±1.7^{b}	353.7	±1.1^{c}
EO9 : 1	−52	±3	−44.9	±0.8^{b}	−28	±5^{abc}	351	±3^{c}
EO6 : 1	−51.40	±0.01	−43.20	±0.01^{c}	−30.00	±0.01^{a}	345.30	±0.01^{d}
EO9 : 1R	−42.1 ± 0.3^{d}				−23.15	±0.05^{c}	350	±6^{cd}
EO6 : 1R	−43.5 ± 0.5^{bc}				−22.85	±0.15^{c}	345.0	±0.2^{d}
EO Sigma	−50.00 ± 0.01				−14.70	±0.01^{d}	383.4	±0.01^{b}

[a] Average values ± standard deviation ($n = 3$). Different superscript letters for statistically significant differences ($p < 0.05$) within rows.

Table 7 Comparison of yield, purity and thermal properties of EO obtained using 9 g and 230 g of HOW with 10% recirculated EO [a]

	Lab scale		Scale-up	
	Mean	SD	Mean	SD
% yield (w/w)	96.35	0.01	97.5	1.8
EO purity (%)	86.16	0.04	86.16	0.06
Crystallisation (°C)	−43.5	0.5	−44.0	0.2
Melting (°C)	−22.85	0.15	−23.4	0.2
Degradation (°C)	345	0.2	343	1.4

[a] Average values ± standard deviation ($n = 3$).

The reported findings suggest that the methodology holds strong potential for adaptation to industrial-scale production of EO from HOW, without compromising product yield, purity, or other key physical characteristics.

Conclusions

High Oleic Waste (HOW) was used in this work to produce ethyl oleate through an alkaline catalysed process of ethanolic transesterification of its triglycerides. Preliminary analysis confirmed that HOW essentially consisted of triglycerides (99.50 ± 0.01%), with the majority being oleic acid the fatty acid (84.767 ± 0.006%).

To achieve a more sustainable process, some variables such as the ethanol : HOW ratio and the introduction of recirculated EO were tested. The effect of each variable was evaluated by using the reaction yield, the purity of the resultant sample in EO and the conversion rate. The optimal conditions determined for the transesterification of HOW into EO were a 6 : 1 mass ratio of ethanol : HOW (w/w), a 10% (w/w) addition of recirculated EO, 1% (w/w) of NaOH towards HOW as the catalyst, at 70 °C for 3 h. Under these conditions, the highest mass yield (96.35 ± 0.006%), EO purity- (86.16 ± 0.02%) and conversion rate (0.87 ± 0.01) were accomplished. These obtained results also point towards a strong potential for adaptation to industrial-scale production.

By optimizing the synthesis process and demonstrating the potential of transforming HOW, this study contributes to the expanding knowledge base in waste valorisation and sustainable production methods within the field of food science. The proposed procedure offers a sustainable and eco-friendly approach to produce EO as a value-added ingredient, encompassing diverse applications in food production, the cosmetic industry, medicine, and biodiesel production, without competition with the food chain.

In this case, since the recovered material (*i.e.* HOW) was a vegetable oil, according to the obtained composition results, it is expectable that the concentrations will remain within levels close to those reported here. It must be considered that this research assayed one particular high-oleic waste source. Therefore, other sources may differ in lipid composition, storage and processing history, which could affect the yield and purity of the final EO.

In the current research, the low FFA content in HOW suggests that the process for which it is primarily used has little impact on the lipid compounds. This work is exploratory research focusing on understanding the lipid nature of HOW, identifying valuable and promising molecules within the framework of circularity, and developing a process able to achieve higher yields and purities than previously reported methods. An additional aim was to ensure that the process could be performed in any research or manufacturing facility. These first steps and the corresponding results highlight the feasibility of these objectives. While the low FFA content, absence of unknown lipophilic molecules during the assayed tests, and the general suitability of EO for human consumption are promising indicators, future work should complement these findings with studies on chemical stability and biocompatibility of the synthesized EO to definitively demonstrate its suitability for food applications.

Data availability

All data regarding this research work are available through the manuscript.

Author contributions

Ana Soares: methodology, investigation, data curation, writing – original draft preparation, writing – review and editing. Ana Luiza Fontes: formal analysis, investigation, writing – review and editing. Francisca Teixeira: investigation, writing – review and editing. Paula Costa: investigation, writing – review and editing. Lígia Pimentel: methodology, investigation, data curation, writing – review and editing, supervision. Manuela

Pintado: funding acquisition. Luis Miguel Rodríguez-Alcalá: conceptualization, methodology, data curation, writing – review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declared that this work was performed without any commercial or financial relationships, and thus there is no conflict of interest.

Acknowledgements

This work was supported by Amyris Bio Products Portugal Unipessoal Lda and Escola Superior de Biotecnologia—Universidade Católica Portuguesa through the Alchemy project, Capturing high value from industrial fermentation bio products (POCI-01−0247-FEDER-027578). The authors would also like to acknowledge the scientific collaboration under the FCT project UID/Multi/50016/2019.

References

1 B. A. F. Gomes, A. C. S. Alexandre, G. A. V. de Andrade, A. P. Zanzini, H. E. A. de Barros, L. M. dos, S. Ferraz e Silva, P. A. Costa, E. V. de and B. V. Boas, *Food Chem. Adv.*, 2023, **2**, 100304.
2 B. Pace and M. Cefola, *Foods*, 2021, **10**, 719.
3 S. K. Amit, M. M. Uddin, R. Rahman, S. M. R. Islam and M. S. Khan, *Agric. Food Secur.*, 2017, **6**, 51.
4 K. An, M. Fu, H. Zhang, D. Tang, Y. Xu and G. Xiao, *J. Food Sci. Technol.*, 2019, **56**, 783–791.
5 S. V. Jangam, *Drying Technol.*, 2011, **29**, 1343–1357.
6 T. Y. Tunde-Akintunde and T. J. Afolabi, *J. Food Process Eng.*, 2010, **33**, 649–660.
7 H. T. Sabarez, in *Modeling Food Processing Operations*, Elsevier Inc., 2015, pp. 95–127.
8 I. Doymaz, *J. Food Eng.*, 2004, **64**, 465–470.
9 I. Doymaz and M. Pala, *J. Food Eng.*, 2003, **60**, 125–130.
10 I. Doymaz and M. Pala, *Nahrung - Food*, 2003, **47**, 304–308.
11 M. Pala, T. Mahmutoğlu and B. Saygi, *Nahrung - Food*, 1996, **40**, 137–141.
12 M. L. Foresti and M. L. Ferreira, *Catal. Today*, 2005, **107–108**, 23–30.
13 D. Bolonio, M. J. García-Martínez, M. F. Ortega, M. Lapuerta, J. Rodríguez-Fernández and L. Canoira, *Renew Energy*, 2019, **132**, 278–283.
14 V. R. Umrigar, M. Chakraborty, P. A. Parikh and H. P. Kohli, *Energy Nexus*, 2022, **7**, 100127.
15 A. Hykkerud and J. M. Marchetti, *Biomass Bioenergy*, 2016, **95**, 340–343.
16 M. José da Silva and A. Lemos Cardoso, *J. Catal.*, 2013, **2013**, 1–11.
17 T. Pinnarat and P. E. Savage, *J. Supercrit. Fluids*, 2010, **53**, 53–59.
18 M. M. Rahman, T. Hassan, M. F. Rabbi, M. F. Shakil and M. A. Khan, in *AIP Conference Proceedings*, American Institute of Physics Inc., 2021, vol. 2324.
19 R. Guzatto, D. Defferrari, Q. B. Reiznautt, Í. R. Cadore and D. Samios, *Fuel*, 2012, **92**, 197–203.
20 G. Anastopoulos, Y. Zannikou, S. Stournas and S. Kalligeros, *Energies*, 2009, **2**, 362–376.
21 M. F. Ortega, D. Donoso, H. Bousbaa, D. Bolonio, R. Ballesteros, M.-J. García-Martínez, M. Lapuerta and L. Canoira, *Waste Biomass Valorization*, 2021, **12**, 2303–2310.
22 A. Demirbas, *Energy Convers. Manage.*, 2008, **49**, 125–130.
23 P. R. Yaashikaa, P. Senthil Kumar and S. Varjani, *Bioresour. Technol.*, 2022, **343**, 126126.
24 S. Negi, L.-S. Fan, H. Kim, T. Hidaka, A. Rani and S.-Y. Pan, *J. Agric. Food Res.*, 2025, **21**, 101925.
25 FAO, FAOSTAT database, https://www.fao.org/faostat/en/#home, accessed 13 May 2025.
26 T. D. Alcock, D. E. Salt, P. Wilson and S. J. Ramsden, *Sci. Total Environ.*, 2022, **829**, 154539.
27 F. Chamorro, M. Carpena, M. Fraga-Corral, J. Echave, M. S. Riaz Rajoka, F. J. Barba, H. Cao, J. Xiao, M. A. Prieto and J. Simal-Gandara, *Food Chem.*, 2022, **370**, 131315.
28 L. L. Pimentel, A. L. Fontes, A. M. Gomes and L. M. Rodríguez-Alcalá, *MethodsX*, 2015, **2**, 475–484.
29 S. Abreu, A. Solgadi and P. Chaminade, *J. Chromatogr. A*, 2017, **1514**, 54–71.
30 F. S. Teixeira, S. S. M. P. Vidigal, L. L. Pimentel, P. T. Costa, D. Tavares-Valente, J. Azevedo-Silva, M. E. Pintado, J. C. Fernandes and L. M. Rodríguez-Alcalá, *Pharmaceuticals*, 2021, **14**, 583.
31 R. Homan and M. K. Anderson, *J. Chromatogr. B:Biomed. Appl.*, 1998, **708**, 21–26.
32 F. S. Teixeira, P. T. Costa, S. S. Vidigal, M. Pintado, L. L. Pimentel and L. M. Rodríguez-Alcalá, *ACS Sustain. Chem. Eng.*, 2023, **11**, 13415–13428.
33 G. Socrates, *Infrared and Raman Characteristic Group Frequencies. Tables and Charts*, London, 3th edn, 2001.
34 A. L. Fontes, L. Pimentel, L. M. Rodríguez-Alcalá and A. Gomes, *Sci. Rep.*, 2018, **8**, 15591.
35 F. Cataldo, *Ozone:Sci. Eng.*, 2015, **37**, 431–440.
36 S. Niu, Y. Zhou, H. Yu, C. Lu and K. Han, *Energy Convers. Manage.*, 2017, **149**, 495–504.
37 S. Liu, T. McDonald and Y. Wang, *Fuel*, 2010, **89**, 2735–2740.
38 P. Castro-Gómez, J. Fontecha and L. M. Rodríguez-Alcalá, *Talanta*, 2014, **128**, 518–523.
39 J. M. Encinar, N. Sánchez, G. Martínez and L. García, *Bioresour. Technol.*, 2011, **102**, 10907–10914.
40 S. Hazarika, P. Goswami, N. N. Dutta and A. K. Hazarika, *Chem. Eng. J.*, 2002, **85**, 61–68.
41 A. Martínez-Ruiz, H. S. García, G. Saucedo-Castañeda and E. Favela-Torres, *Appl. Biochem. Biotechnol.*, 2008, **151**, 393–401.
42 Y. Zheng, Y. Zheng, S. Yang, Z. Guo, T. Zhang, H. Song and Q. Shao, *Green Chem. Lett. Rev.*, 2017, **10**, 202–209.