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Characterization of *Annona cherimola* Mill. Seed Oil from Madeira Island: a Possible Biodiesel Feedstock

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Abstract The possibility of using Annona seed oil as an added value product, namely as a source of biodiesel, is explored. Milled Annona seeds were extracted with hexane at room temperature (72 h) and at solvent boiling point (6 h). Oil content was found to be 25 and 22.4% respectively. The oil was characterized in terms of lipid composition (HPLC–APCI–MS and ^{13}C NMR), resistance to oxidation and acidity index. FAME composition was determined by GC–MS and five major peaks were identified. Production of biodiesel from Annona's seed oil was achieved by base-catalyzed transesterification. Density, viscosity, refraction coefficient, acid value, cold filter plugging point, cloud point and oxidation stability were measured. The iodine value and the "apparent cetane number" were calculated. Density, viscosity, acid value, iodine value, cold filter plugging point and cloud point were within EN14214 specifications and the calculated "apparent cetane number" was also indicative of a suitable product.

Keywords Annona seed oil · Residue valorization · Triacylglycerols · Fatty acid · Methyl ester · Biodiesel production

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Introduction

Residual agricultural products and food-processing by-products or wastes are often considered a problem. After processing, a large amount of plant material often remains without any application [1]. The conversion of such materials into valuable resources can be a good contribution to a reduction in residues. In this sense, residue valorization has become of great interest from an economical point of view.

Fruit seeds are often considered as waste products in some industries [2–4] and several studies have been carried out to evaluate their suitability as a source of biodiesel. This application can generate a great economical interest since industrial waste is then converted into a useful by-product. On the Madeira Island, *Annona cherimola* Mill. is consumed as fresh fruit and part of the production is exported. Yet, since this is a very sensitive fruit, approximately half of the total production is lost and 500 tons of waste are produced every year and this is usually disposed off or used as fertilizer. Seeds represent about 25% of the residue weight and may be used as a source of biodiesel. Seeds of other Annona species (*Annona squamosa* and *Annona muricata*) have been surveyed for their possible use as a source of biodiesel [5]. *A. cherimola* fruit production is largely developed in some Latin American countries, therefore large quantities of residue might be available.

Biodiesel (fatty acid alkyl esters) produced from vegetable oils has been considered a viable alternative to fossil diesel. As dedicated agro-production can have disastrous economical consequences there has been great discussion about using soils for energy crops instead of using them for feeding purposes. Therefore waste cooking oils and non-edible vegetable oils have been considered as a potential

source of alternative fuels [3, 4], since they do not compete with food crops for the occupation of the soil. Waste seed oil can be considered as a non-edible vegetable oil, although in some African or Middle East countries pumpkin and melon seeds (waste products after the removal of the pulp and peel) are used to extract cooking oils [1].

Oil characterization is essential to evaluate its potential applications, such as cosmetics, nutrition [6] or fuel [7] industries. Chromatographic methods with mass spectrometric detection are well established to determine lipid composition. GC–MS is widely used to determine fatty acid (FA) composition, mainly as their methyl esters (FAMEs), and HPLC–MS has been used for triacyglycerol (TAG) characterization. ^{13}C NMR can also be used to quantify free fatty acids (FFA) and partial acylglycerols, which are normally present as traces [8].

Regarding fuel applications, the measurement of the oil's stability to oxidation and its acidity index can be screening methods to predict the suitability of the oil as a raw material for biodiesel production. Oils with low oxidation stability cannot be stored for a long time before conversion and it will probably result in a final product that most certainly is not itself resistant to oxidation processes. On the other hand, high FFA content (thus resulting in high acidity index) are inconvenient for base-catalyzed transesterification processes, since the catalyst is consumed in a saponification reaction and larger amounts of base are needed, lowering biodiesel yield as well as emulsifying the final product hindering glycerol separation [9].

Methods

Materials

Annona seeds were provided by AGRIPÉROLA—Cooperativa Agícola, C.R.L. (Funchal, Portugal). Hexane (95%) and Acetonitrile (LC-MaScan) were purchased from LAB-SCAN Analytical Sciences (Dublin, Ireland), methanol (99.8%), sodium chloride (analytical reagent), sodium hydroxide pellets (analytical reagent) and ethanol absolute (UV-IR-HPLC) from PANREAC (Barcelona, Spain), hydrochloric acid (37%) and diethyl ether (99.5%) from Riedel-de Haën (Seelze, Germany), propan-2-ol (HPLC Gradient grade) from Fisher Scientific (Loughborough, UK), hexane (for HPLC) from Acros Organics (NJ, USA), potassium hydroxide (analytical reagent) and anhydrous magnesium sulfate (analytical reagent) from Merck (Darmstadt, Germany), boron trifluoride methanol complex from BDH (Poole, England), glyceryl trilaurate (≈99%), glyceryl tripalmitate (≈99%) and glyceryl trioleate (>99%) from Sigma (Steinheim, Germany) and glyceryl tristearate (>99%) from Fluka (Steinheim, Germany).

Oil Extraction and Characterization

Extraction Procedures

Dry seeds were ground to a powder in an IKA® Werke (Staufen, Germany) MF10 basic mill. Powdered Annona seeds (400 g) were added to 4 L of hexane and stirred for 72 h at room temperature (≈25 °C). In parallel, an extraction at solvent boiling temperature (6 h) was carried out for comparison. Both hexane extracts were then filtered and evaporated in a rotary evaporator to eliminate solvent.

Determination of the FFA Content by Titrimetry

The acidity index of fresh oil was calculated according to ISO 660 standard method [10].

Oil Resistance to Oxidation

The FT-IR spectrum of the recently extracted oil was registered in a Mattson (Madison, WI, USA) Infinity Series FT-IR using KBr cells, accumulating 32 scans from 4,000 to 600 cm^{-1}, with a scan resolution of 4 cm^{-1}. Fresh oil (10 g) was placed in a covered Petri dish and kept at 70 °C. FT-IR spectra were registered every 24 h as described before.

Induction period and stability curves were determined according to EN14112 [11] procedures in a Metrohm (Herisau, Switzerland) Rancimat® model 743 equipment.

Determination of FA Composition

FA were converted to FAME and determined according to the analytical methods described in regulations EEC/2568/91 [12] and EEC/1429/92 [13] of the European Union Commission for olive oil.

FAME Analysis

The FAME composition was determined by CG–MS [12, 13], using a Varian (Walnut Creek, CA, USA) Star 3400 Cx Series II gas chromatograph equipped with Varian (Walnut Creek, CA, USA) Saturn III mass selective detector and Saturn GC–MS workstation software. A J&W (Rancho Cordova, CA, USA) DB-wax (30 m) column with 0.25 mm (i.d.) and 0.25 mm thickness coating film was used. Operating conditions: injector temperature, 240 °C; initial temperature (column oven), 70 °C; heating ramp, 10 °C/min to 180 °C, 10 min at 180 °C, 10 °C/min to 220 °C and 10 min at 220 °C. The ion trap detector was set

as follows: transfer line temperature 220 °C; manifold and trap temperatures 180 °C; mass range *m/z* 35–350; emission current 15 mA. The electron multiplier was set in the relative mode to the auto tune procedures. All mass spectra were acquired in the electron impact mode ($E_i = 70$ eV; source temperature, 180 °C). The sample injection volume was 1 μL.

Evaluation of the Lipidic Composition by ^{13}C NMR

The oil, without any treatment, was dissolved in $CDCl_3$ and analyzed by ^{13}C NMR. Spectra were recorded on a Bruker (Rheinstetten, Germany) AVANCE 400 II+ operating at 100.61 MHz, equipped with a 5-mm BBO probe. Chemical shifts (δ) are all relative to internal tetramethylsilane. The spectra were recorded at room temperature with a 2-s relaxation decay, a 45° excitation pulse, a 2.36-s acquisition time, a sweep total with of 24,038 Hz and 32-K acquisition points to yield a digital resolution of 0.212 Hz/point [14].

Determination of TAG Structure by HPLC–APCI–MS

The TAG composition was determined using a Dionex (Germering, Germany) UltiMate 3000 series chromatograph equipped with a Phenomenex Gemini C18 column (250 × 3.0 mm, 5 μm particle size) and a Bruker (Bremen, Germany) Esquire 6000 mass detector, using positive-mode atmospheric pressure chemical ionization (APCI) with an ion-trap mass analyzer. The samples and column were kept at 30 °C, and a gradient elution was effected by changing the mobile phase composition from acetonitrile/ethanol (90:10) to 18% acetonitrile in 90 min. The mobile phase flow was 0.7 mL/min. 3 and 0.5% (w/v) solutions of the oil and standards (respectively) dissolved in acetonitrile/propan-2-ol/hexane (2:2:1) were prepared and 20 μL was injected [15].

Biodiesel Production and Characterization

Biodiesel Production Procedure

Freshly obtained oil (50 mL) was heated to 60 °C in a round-bottom flask. Sodium hydroxide (0.175 g) was dissolved in 175 mL of methanol and added to the oil. The reaction was kept under reflux (55–60 °C) for 1 h. The reaction mixture was then transferred to a separator funnel and left to cool down to room temperature. At this point two phases were observed. The biodiesel formed was washed with distilled water (15% of biodiesel's volume) and the aqueous phase was discarded. The biodiesel was then washed with 0.5% aqueous HCl (5% of biodiesel's initial volume) and again with water (10% of the initial volume of biodiesel) to remove soaps, the aqueous phases were discarded in both steps. Finally, the biodiesel obtained was dried with anhydrous magnesium sulfate, filtered and any excess water was evaporated in a rotary evaporator.

Biodiesel Characteristics

The following parameters were determined: density (picnometer method), kinematic viscosity (Ubbelohde capillary viscometer), iodine number (gas chromatography), acid value (titrimetry), cold filter plugging point (CFPP), cloud point, oxidation stability (Rancimat® method) and calculated "apparent cetane number" (Klopfenstein's equation).

Density was measured at 15 °C following the procedures indicated in UNE-EN 14214 [16] and kinematic viscosity was measured at 40 °C, according to the EN ISO 3104 [17] standard method. The iodine number was obtained by gas chromatography, as laid out in UNE-EN 14214 [16]; GC–MS conditions were the same as described for FAME analysis. The procedure for calculating the acid value is the same as indicated for the oil's acidity index [10]. The CFPP was determined according to the EN 116 standard method [18] and the cloud point was determined by the ASTM D 2500 standard method [19]. The oxidation stability procedure was the same as previously described for the oil.

For each individual methyl ester "apparent cetane number" ($I_{cetane,ME}$) can be estimated by using Klopfenstein's equation (Eq. 1) [20]. "Apparent cetane number" can then be predicted using Eq. 2 as the average of the product of each individual methyl ester contribution by its percentage in the mixture [21].

$$I_{cetane,ME} = 58.1 + 2.8 \times \frac{(n-8)}{2} - 15.9 \times DB \quad (1)$$

$$I_{cetane} = \sum X_{ME} \times I_{cetane,ME} \quad (2)$$

where n is the number of carbons in the acyl chain, DB the number of double bonds and X_{ME} the weight percentage of individual methyl ester.

Results and Discussion

The oil content of *A. cherimola* seeds was found to be 25.1% (in terms of neutral lipids), obtained by extraction with *n*-hexane at room temperature, while extraction at boiling temperature afforded only 22.4% of neutral lipids. These quantities are far below canola but above soybean oils, some usual sources of vegetable oil for biodiesel production [22].

Oil Characterization

The acidity number for the freshly extracted oil was 2.2 mg KOH/g. This value is consistent with other raw vegetable oils suitable for biodiesel production. Yet this value should be reduced to values below 1.0 mg KOH/g prior to alkali catalyzed transesterification. The most widely used method to reduce FFA content is the acid catalyzed pre-esterification [23].

The FT-IR spectrum of freshly obtained oil (Fig. 1a) shows a weak band at 3,474 cm^{-1}, due to an overtone band of carbonyl group stretching [24]. The bands at 3,008, 1,653 and 917 cm^{-1}, indicate the presence of *cis* unsaturated FAs and are due respectively to C–H, C=C stretching and C=C deformation (out-of-plane) in olefin groups [25, 26].

The high degree of unsaturation of *A. cherimola* seed oil makes it susceptible to oxidation. Although the traditional methods for establishing the oxidative state of oil and fats are chemical methods based on the measurement of the concentration of the main products generated in the process, such as peroxide value, anisidine value and iodine value, we present some observations based on infrared spectroscopy, since good correlations can be established between this and the traditional processes. FT-IR methods are increasingly being accepted for the evaluation and monitoring of oxidation processes in transformer and motor oils as well as vegetable oils, to replace costly, labor and time consuming chemical methods. These spectrometric methods combine speed of analysis, high sensitivity, precision and reproducibility, and require a minimum amount of sample [27–29].

The comparison of the IR spectra of the oil initially and after 8 and 12 days under oxidative conditions (Fig. 1a, b and c) show some changes that indicate the oxidative process. At approximately 3,500 cm^{-1}, an intense broad band appears after 8 days, indicating the presence of hydroperoxides. This band gets even more intense after 12 days. The disappearance, after 8 days, of the peak at approximately 3,000 cm^{-1} means that the double bonds are fully oxidized. The band corresponding to carbonyl stretching at 1,746 cm^{-1} has a drift to 1,743 cm^{-1} after 12 days. This indicates the formation of FFA, as oxidation product of triglycerides. Finally, the disappearance of the band at 1,119 cm^{-1} means that the degree of saturation rises, since the intensity of this band is inversely proportional to the degree of saturation [25, 26]. A new band is observed at 986 cm^{-1}; this is absent from the spectrum of the freshly obtained oil and appears during the oxidation process. This band has been associated with bending vibrations of CH *trans–trans*-conjugated unsaturated FAs.

The standard test method to determine oxidation stability for biodiesel products is the Rancimat® method. The test was conducted for comparison with the previous results and the induction period for Annona seed oil was 3.96 h.

These results indicate that the oil should not be kept stored for a long period, thus avoiding oxidation and consequently the increase of the FFA content, which will decrease the yield when converting the oil into biodiesel.

FA Composition

Five major peaks were found in the GC–MS chromatogram of FAME. The peaks were identified respectively as C16:0 (RT = 22.36 min; $M^+ = 270$ *m/z*), C18:0 (RT =

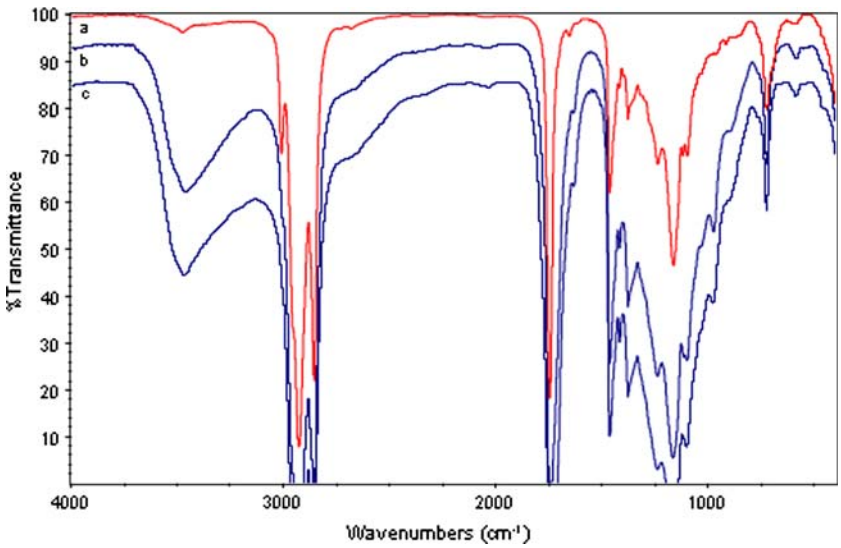

Fig. 1 FT-IR spectra of **a** freshly obtained oil and after **b** 8 days and **c** 12 days under oxidative conditions

28.48 min; $M^+ = 298$ *m/z*), C18:1 (RT = 29.21 min; $M^+ = 296$ *m/z*), C18:2 (RT = 30.44 min; $M^+ = 294$ *m/z*) and C18:3 (RT = 32.19 min; $M^+ = 292$ *m/z*), and their relative abundance is presented in Table 1.

When compared with some leading sources [30] of biodiesel (Table 1) Annona seed oil has a higher content in palmitic acid, almost equal parts of oleic and linoleic acids (much different to soybean, rapeseed and sunflower oils [31]) and a low content in linolenic acid. This composition indicates that Annona seed oil is a potential biodiesel source and as stable to oxidation as soybean and sunflower oils.

^{13}C-NMR Analysis

The spectrum of annona seed oil (Fig. 2) shows mainly the characteristic signals of TAG. 1,3-DAG signals are of very low intensity (so low that the signals of the glyceride carbons at ca. 65 ppm cannot be integrated) and 1,2-DAG (signals in the glyceride carbon region at ca. 72 ppm) and FFA (carbonyl resonance at ca. 176 ppm) are detectable as traces [8].

Although ^{13}C NMR can provide essential structural information about TAG, concerning the structure of the acyl groups and their distribution in the glycerol backbone, it was used in this study mainly to confirm that Annona seed oil contained essentially TAG.

TAG Structure

Eleven peaks, affording 17 TAG, were identified in the HPLC–APCI–MS chromatogram of the oil (Fig. 3). TAG elution in RP-HPLC is affected by both the combined number of carbon atoms in the acyl chain (ACN acyl carbon number) and the number of double bonds, *n*, in the molecule; as some critical groups (TAG with the same equivalent carbon number ECN = ACN − 2*n*) are usually difficult to separate [32], some peaks were a result of a co-eluted mixture of up to three TAG.

Table 1 FA composition of Annona seed oil and other vegetable oils used as biodiesel sources (%)

	Annona seed oil	Soybean oil[a]	Rapeseed oil[a]	Sunflower oil[a]
Palmitic acid (C16:0)	19.99	11.75	3.49	6.08
Stearic acid (C18:0)	4.16	3.15	0.85	3.26
Oleic acid (C18:1)	38.58	23.26	64.40	16.93
Linoleic acid (C18:2)	35.97	55.53	22.30	73.73
Linolenic acid (C18:3)	1.31	6.31	8.23	0.00

[a] Ma and Hanna [31]

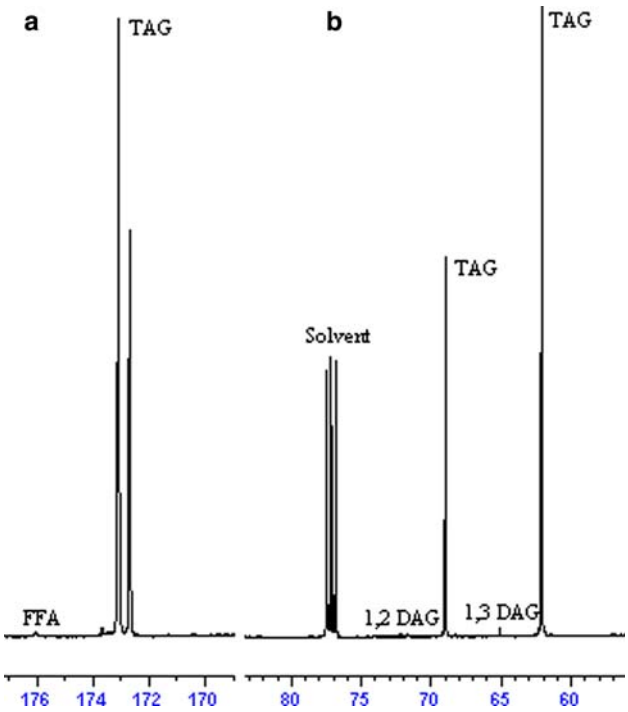

Fig. 2 ^{13}C-NMR spectrum (100.61 MHz) of **a** the carbonyl carbons and **b** the glycerol carbons of Annona seed oil

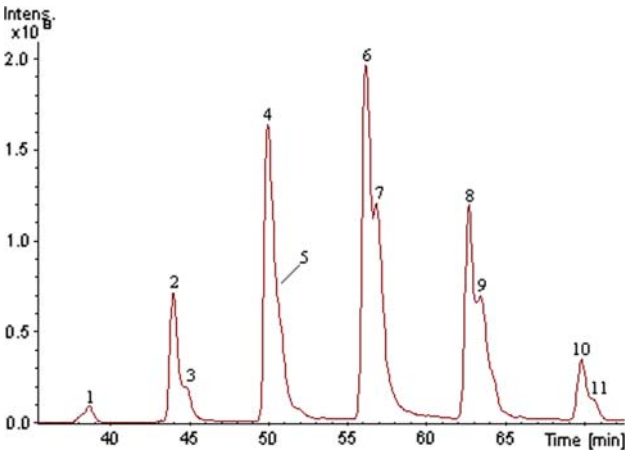

Fig. 3 Reconstructed ion chromatogram of TAG from Annona seed oil achieved by HPLC–APCI–MS

The identification of the molecular species was mainly based on the mass spectral data, the $[M+H]^+$ ion provided molecular weight (MW) information, whereas the information on the FA residues was given by the DAG fragment ions ($[M\text{-}RCOOH]^+$ ion). Positional identification of the FA in the glycerol moiety were possible taking into account some considerations [14]: seed oils normally have PUFA in the *sn*-2 position and the differences between *sn*-1 and *sn*-3 are very small, although less abundant FA tend to appear in the *sn*-3 position; the position of the FA in the glycerol backbone affects the elution order, with

unsaturated FA in the *sn*-2 position causing the TAG to elute before the TAG with the same FA in the *sn*-1 or *sn*-3 positions; positional isomers can be identified from the relative intensities of the DAG fragment ions, as the least abundant DAG fragment ion is due to the loss of the FA from the *sn*-2 position. This last consideration is quite useful to differentiate between mixed symmetric and mixed asymmetric TAG (ABA and AAB type of TAG) because the AA/AB ratio of the DAG fragment ions is extremely different, being lower than 1 when B is in the *sn*-2 position.

The suggested FA combinations (Table 2) show that the four main types of TAG were distinguished: AAA, monoacid type (LLL and OOO); AAB, mixed asymmetric type (PLL, OLL, OOL, LLS and OOS); ABA, mixed symmetric type (LLnL, LOL, OLO, OPO and OSO); ABC mixed asymmetric type (OLnL, PLO, OLS, OLA and POS).

Conversion of the Oil into Biodiesel and Its Properties

The amount of FAME obtained by this method was 75.88 g/100 g of seed oil.

EN 14214 [16] establishes the requirements for biodiesel quality. The parameters determined and calculated for biodiesel quality are shown in Table 3.

The cetane number is related to the ignition of the fuel and a low cetane number indicates that the combustion is not complete. Consequently, part of the fuel remains in the combustion chamber and it will produce more energy than needed when burned, wearing out the engine. As the equations used to predict cetane number are not appropriate to biodiesel, some correlations were developed for methyl esters [19]. A fuel with high "apparent cetane number" (no. 2 diesel fuel has a cetane number of 46) is better for the cold start of the engine, allows a quick warming of the engine and reduces noise and gas emissions to the atmosphere [33]. Biodiesel produced from Annona seed oil has a calculated "apparent cetane number" of 53 (EN 14214 [16] establishes a minimum value of 51).

The iodine value evaluates the number of double bonds, quantifying the unsaturation degree of the fuel, indicating its tendency to oxidation. The iodine number of the biodiesel produced was below the EN 14214 [16] upper limit.

The acidity value determined was also below the EN 14214 [16] upper limit, indicating a low quantity of FFA in the biodiesel produced.

Table 3 Properties of the biodiesel produced

	Biodiesel	EN 14214 limits
Density, 15 °C (g/cm^3)	0.871	0.860–0.900
Viscosity, 40 °C (cSt)	4.4	3.5–5.0
Acid value (mg KOH/g)	0.3	<0.5
Iodine number	99[a]	<120
Oxidation stability, 110 °C (h)	1.17	>6
CFPP (°C)	−5	
Cloud point (°C)	1	–
"Apparent cetane number"	53[a]	>51

[a] Calculated

Table 2 Molecular species identification of the TAG of Annona seed oil

Peak #	RT (min)	ECN	TAG	$[M+H]^+$	DAG	$[M-R_1COO]^+$	DAG	$[M-R_2COO]^+$	DAG	$[M-R_3COO]^+$
1	38.5	40	LLnL	877.8	LnL	597.5	LL	599.6		
2	43.9	42	LLL	879.8	LL	599.6				
3	44.7	42	OLnL	879.9	LnL	597.6	OL	601.6	OLn	599.6
4	49.8	44	LOL	881.9	OL	601.6	LL	599.5		
5	50.6	44	PLL	855.9	LL	599.6			PL	575.6
			OLL	881.9	LL	599.6			OL	601.6
6	56.0	46	OLO	883.9	OL	601.6	OO	603.6		
7	56.8	46	PLO	857.9	LO	601.6	PO	577.6	PL	575.6
			OOL	883.9	OL	601.6			OO	603.6
			LLS		SL	603.6			LL	599.6
8	62.6	48	OOO	885.9	OO	603.6				
9	63.1	48	OPO	859.8	PO	577.6	OO	603.6		
			OLS	885.9	LS	603.6	OS	605.6	OL	601.6
10	69.7	50	OSO	887.9	OS	605.6	OO	603.6		
			OLA	913.9	LA	631.7	OA	633.7	OL	601.6
11	70.5	50	POS	861.9	OS	605.6	PS	579.6	PO	577.5
			OOS	887.9	OS	605.6			OO	603.6

L linoleic, *Ln* linolenic, *O* oleic, *P* palmitic, *S* stearic, *A* arachidic, *ECN* equivalent carbon number, *RT* retention time

The FAME composition of the biodiesel produced is very similar to the FAME composition of the oil (Table 1), thus indicating that the method for biodiesel production does not promote isomerization or hydrogenation. The predominance of unsaturated FAME makes the biodiesel susceptible to oxidation, but it also balances the viscosity of the fuel, allowing an optimal flow throughout the system. Due to the higher content of saturated FAs (when compared to other oils commonly used for the production of biodiesel) it would be expected that FAME derived from *A. cherimola* oil would have worse low temperature properties. The results show that these properties are in fact similar to those exhibited by biodiesels produced from soybean or rapeseed oils [34].

Conclusions

Oil extraction procedures demonstrated that percolation at room temperature yields more oil than at solvent boiling temperature (25.1 vs. 22.4%). Although the energy balance favors room temperature, it must be taken into account that time is also a very important parameter.

The lipid content of *A. cherimola* Mill. seeds is suitable for making this a promising source of biodiesel as confirmed by the evaluated parameters of the methyl esters. The high degree of unsaturation (all TAG include oleic and/or linoleic acids and FAME analysis confirmed the predominance of these FA) makes the biodiesel produced from this oil susceptible to oxidation, but it also improves (reduces) its viscosity. The low oxidation stability can be overcome by introducing additives into the final product.

Biodiesel production can be a contribution to avoid waste disposal. However, this is a seasonable crop and it must be accompanied by other sources of biodiesel, such as waste cooking oil, another residue with a high economical value.

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