

Lemon seed oil: An alternative source for the production of glycerol-free biodiesel

Carmelo Drago[†] , Raffaele Morrone[†] , Nicola D'Antona , Giuseppe Ruberto , Edoardo Napoli , Istituto di Chimica Biomolecolare—Consiglio Nazionale delle Ricerche, Catania, Italy

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Abstract: The aim of this study was the evaluation of two different procedures for the *one-pot* transformation of oil extracted from *Citrus limon* seeds to a mixture of fatty acid methyl esters (FAMES) and glycerol derivatives for application as a potential biofuel. Lemon seed oil was obtained by Soxhlet extraction. The first procedure was realized by efficient irreversible transesterification of the oil in hexane by lipase B from *Candida antarctica* (Novozym 435[®]) using dimethyl carbonate as the alcohol donor. For the realization of the second methodology an acid-catalyzed transformation was carried out, dissolving the seed oil in methyl *tert*-butyl ether in a microwave tube using Amberlyst[®]-36 *dry form* catalyst. Both procedures, in optimized conditions, led to the complete conversion of the triglycerides to give the corresponding FAMES and a mixture of glycerol derivatives. The absence of *free* glycerol in the final mixtures makes the two herein described procedures considerably advantageous in terms of both cost and sustainability since they enable performance of the production of FAMES without requiring steps to remove glycerol. These final mixtures may be used in the energy chain and exploited as biofuels. © 2022 The Authors. *Biofuels, Bioproducts and Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: biodiesel; *Citrus limon* seeds; irreversible transesterification; heterogeneous catalysis; sustainability; glycerol free

Introduction

The genus *Citrus* with its 1300 species can be considered as one of the largest fruit crops worldwide, with a global year production in 2019 of 143 755.6 million tons (2864.97 million tons of which were in Italy), 11% of which comprised lime and lemon.¹ Southern Italy plays an important role, covering up to 6.5% of world production.

About 33% of the whole world production is destined for processing for the production of juice and essential oils. This turns into a huge production of *Citrus* processing waste (CPW), representing peel, seeds and residual pulp, amounts to 50–60% of the processed fresh fruit.² Since the origins of the *Citrus* processing industry, CPW has been used mainly as animal feed.³ However, its use is limited by the high degree of humidity, which makes transfers from production sites

Correspondence to: Edoardo Napoli, Istituto di Chimica Biomolecolare—Consiglio Nazionale delle Ricerche, Via Paolo Gaifami 18, 95126 Catania, Italy. E-mail: edoardomarco.napoli@cnr.it

[†]These authors contributed equally.

In memory of Giovanni Nicolosi.

to those of use expensive. Moreover, the variable availability linked to its seasonal production leads to costs to have it available throughout the year.⁴ *Citrus* processing waste can also be used for agronomic purposes as a soil conditioner or as a base for the production of compost.

The use of CPW for biogas production has been investigated. However, both its rapid acidification and inhibiting effect owing to the presence of limonene remain problems to be overcome.⁵ Recently novel extraction/purification techniques for obtaining bioactive compounds such as polyphenols, fibers, pectins, carbohydrates, sugars, citric acid, vitamins and limonoids have been developed, but at present they manage to transform only limited quantities of product. For these reasons, much attention is still being paid to the design of new forms of exploitation of this waste, using innovative and environmentally sustainable strategies.^{6,7} *Citrus* processing waste from lemon can contain up to 5% by weight of seeds,⁸ which can be considered as an interesting source of quality seed oil⁹ and different extraction methods have been investigated. The yields and properties of cold pressed vs. solvent-extracted *Citrus limon* L. seed oils were recently compared¹⁰ as well as supercritical carbon dioxide-extracted oil.^{11,12}

Citrus seed oil has experienced increasing interest in relation to its chemical composition, but especially for its content of fatty acids and in particular for the high content of polyunsaturated fatty acids that makes it a potential and interesting source of vegetable oil.¹³ Conversely, the presence of substances with a strong bitter taste, such as flavonoids and limonoids,¹⁴ is a serious limiting factor for direct human consumption.¹⁵ For this reason, it is necessary to hypothesize a different use for this type of oil, such as using as a high added value active ingredient in the nutraceutical and cosmetic field. On the other hand, in an era in which the substitution of fossil fuel sources pushes us toward the search for new renewable energy sources, it is reasonable to consider non-edible seed oils also as a potential source for biodiesel production.^{16,17}

Biodiesel is an advanced and renewable clean-burning biofuel alternative to conventional petroleum diesel because it can be used directly in conventional diesel engines.^{18,19} The performance, efficiency and long-term preservation of diesel engines depend on the lubricity of the fuel itself. In traditional petroleum diesel the lubricity is due to the content of polycyclic aromatic hydrocarbons; however, their combustion generated pollutant emissions.^{20,21} Despite having a negligible aromatic content, biodiesel has a good lubricating power and an high cetane number, which are parameters linked to its high combustion efficiency with low emission from polycyclic aromatic hydrocarbon

combustion.^{22–24} Furthermore, biodiesel has a low sulfur and nitrogen content, which is in line with the mandatory environmental specifications for fuels introduced by the EU Directives in terms of low emissions of sulfur and nitrogen oxide (NO_x) pollutants.^{20,25–28}

Biodiesel consists of fatty acid alkyl esters produced by the catalytic transesterification of triglycerides with an alcohol, such as methanol or ethanol. Most current industrial transesterification processes use alkaline chemical catalysts that are inexpensive and give high levels of conversion in short reaction times, but are not directly suitable for vegetable oils with high contents of free fatty acids, leading to the formation of soaps and causing both the catalyst deactivation (neutralization) and serious problems concerning the *recovery* of the final desired product. Usually, for such cases, it is necessary to use a two-step process: the first step converts the free fatty acids by acid catalysis to the corresponding fatty acid methyl esters (FAMES) and then the second leads to transesterification through a basic catalysis.^{29–34} A further issue to address is the inevitable production of glycerol as the co-product (about 10% of produced biodiesel), which must be removed and whose formation impacts on the cost of the entire process.^{35,36}

Enzymatic transesterification eliminates several of these drawbacks and yields a high-purity product, with the catalyst often readily recovered and re-used.^{37,38} The use of non-edible oils for biodiesel production from a circular economy point of view has additional benefits such as treatment of a waste product and the efficient use of a resource.

The aim of this study was to develop two different strategies, an enzymatic and a chemical one, for the production of glycerol-free biodiesel, using as starting material *C. limon* L. seeds oil.

Experimental section

Plant material

Seeds of *C. limon* L. were kindly supplied by Ditta Lucchesi, Aci Catena (CT), Italy.

Chemicals

All purchased chemical reagents were of analytical grade and used as received. Lipase B from *Candida antarctica* (Novozym® 435; CALB) and Amberlyst®-36 *dry form* were kindly gifted by DowDuPont™ (thanks to Dr Klaus-Dieter Topp and Dr Antonio Arzu), and diethyl carbonate was supplied by Fluka.

Seed oil Soxhlet extraction

Citrus limon seeds were washed, finely crushed and then freeze-dried. The dry material (100 g) was subjected to Soxhlet extraction with hexane (1 L) for 3 h. The solvent was then removed with a rotating evaporator providing 19.34 g of *C. limon* seeds oil.

Transesterification of seed oil by solid acid catalysis

A sample of the selected oil extracted from *C. limon seeds* (1.5 g) was dissolved in methyl *tert*-butyl ether (MTBE; 1.6 mL, d 0.744 mg/mL; 24.5 mmol). It was poured into a microwave tube and Amberlyst®-36 *dry form* (30 mg) was added to the resulting solution. The reaction mixture was irradiated in a microwave reactor, pre-set at 20 W (in the preferred conditions, the maximum temperature limit was set at 130°C) until the complete conversion of the substrate (3 h). The trend of the reaction was monitored by the HPLC method as previously described.

The GC analysis of the final mixture showed the FAME composition and the presence of both di- and mono-*tert*-butyl glycerol ethers.

Enzymatic transesterification of seed oil by an irreversible procedure

To a 12 mL vial containing 2.5 mL of hexane were added 50 mg of seed oil (0.55 mmol) and 10 μ L (0.11 mmol) of dimethyl carbonate as the alcohol donor. Then 25 mg of Novozym® 435 and 1 μ L of methanol were added to start the reaction. The mixture was kept in shaker at 45°C and 300 rpm. The course of the reaction was monitored with TLC analysis of the reaction mixture using as eluent a mix of hexane–ethyl acetate with a 9:1 ratio. After 15 h, the complete conversion of triglycerides to the corresponding FAMEs was observed and the reaction was stopped by filtering off the lipase. The GC analysis of hexane solution showed the FAME composition.

HPLC procedure for monitoring the biocatalyzed transesterification

The course of the reaction was carried out with an HPLC procedure using a Varian 9010 instrument equipped with an Alltech 3300 evaporative light scattering detector (ELSD). A Luna C₁₈ column from Phenomenex (250 \times 4.6 mm, 5 μ m particle size) was used for the separation of triglycerides and FAMEs. The HPLC conditions were as follows: eluent A, MeOH; eluent B, CH₂Cl₂; gradient: 0–3 min (A–B/80:20), 3–18 min (A–B/30:70), 18–23 min (A–B/30:70); at a flow of

1 mL/min. The ELSD was set to a probe temperature of 40°C, a gain of 16, and the nebulizer gas nitrogen adjusted to 1.5 L/min.

GC analysis of FAMEs

An aliquot of the reaction mixture was injected on a Shimadzu GC-17A equipped with a fused-silica capillary column from J&W Scientific (INNOWAX, 30 m, 0.25 mm, 0.25 μ m); nitrogen was the carrier gas. The reaction mixture was analyzed using the following temperature program: 160°C for 1 min; 160–250°C at 2°C/min; and 250°C for 5 min. The injector and detector temperatures were 250 and 280°C, respectively. The identification of different FAMEs obtained by the enzymatic transesterification process was achieved by referring to the chromatograms of standard compounds.

Results and discussion

FAME production by irreversible esterification with *C. antarctica* lipase B (Novozyme 435)

Biodiesel is becoming ever more attractive because of its environmental benefits and the fact that it is made from renewable resources. Classically, the literature reports four primary ways to make biodiesel from vegetable oils: direct use by blending or microemulsions, and transformations by thermal cracking (pyrolysis) or transesterification.^{39,40} The most commonly used method is the basic- or acid-catalyzed transesterification of vegetable oils (and animal fats), which is affected by many parameters such as the molar ratio of glycerides to alcohol, the reaction temperature, the reaction time, the need for catalysts and the free fatty acid and water content of the lipids.

In general, biodiesel production *via* this commonly established chemical transesterification is an energy-intensive process, with difficult downstream operations owing to the formation of soaps when operating in basic conditions, and the further disadvantage of being a reversible process (Fig. 1), which does not allow exhaustive conversion.

An alternative to chemical transesterification is represented by the enzyme-catalyzed process that is often considered

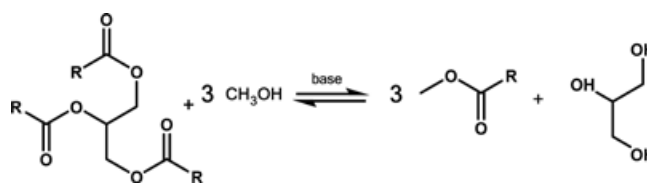


Figure 1. Progress of triglyceride *trans*-esterification by conventional chemical transformation.

more advantageous than the other methods because of its mild reaction conditions, higher quality of products, no wastewater generation, no saponification and easy product recovery. The main component of this reaction is an enzyme called lipase which can catalyze the *trans*-esterification of a wide variety of substrates, including free fatty acids. Biodiesel preparation catalyzed by the enzyme is affected by many factors, such as lipase specificity and immobilization, the oil composition and purity, the oil to acyl acceptor molar ratio, acyl acceptors, temperature and the water content, and while it remains an equilibrium process, we have developed strategies that allow to lipase biocatalyzed esterifications of fatty acids to be carried out in an irreversible way through the use of specific alcohol donors, such as alkyl carbonates or trialkyl orthoformates, that consume the water that originated in the reaction, pushing the equilibrium of the reaction toward an irreversible trend.^{41–43}

Analogously in this work, a procedure for the transesterification of triglycerides of lemon seed oil with dimethyl carbonate as an alcohol donor and immobilized lipase B from CALB as the catalyst has been applied. The aforesaid process is irreversible because the intermediate compound, namely carbonic acid monoacyl ester, immediately decomposes to carbon dioxide and an alcohol (Fig. 2), and no glycerol by-product is obtained since it is transformed into glycerol carbonate that is completely soluble in the final mixture, being consequently homogeneous and representing an interesting potential biodiesel additive.⁴⁴

An HPLC procedure was used to monitor the progress of the transesterification reaction. Aliquots of the reaction medium were taken at different times and analyzed by HPLC. After 24 h, 98% of the triglycerides had been converted to FAMES (Fig. 3).

The biodiesel characterization by GC analysis (Fig. 4) showed the following relative concentrations of FAMES: 21.4% palmitic-Me; 3.7% stearic-Me; 29.2% oleic-Me; 36.3% linoleic-Me; 8.9% linolenic-Me; and 0.5% arachidic-Me. The biocatalyst used for the process of transesterification kept its activity unchanged for five cycles. It is interesting to note that the unsaturated fraction represents 74.4% of the fatty acids, making this oil very interesting and potentially valuable also

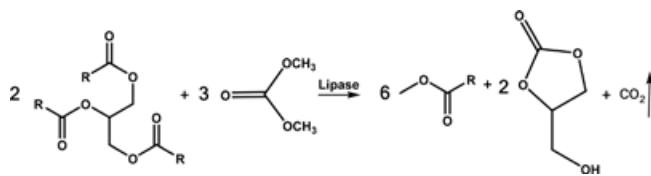


Figure 2. Irreversible triglyceride *trans*-esterification by lipase-catalyzed transformation.

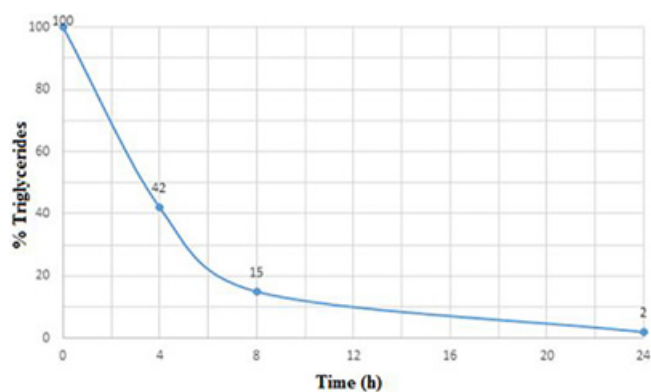


Figure 3. Progress of triglyceride transesterification by enzymatic transformation.

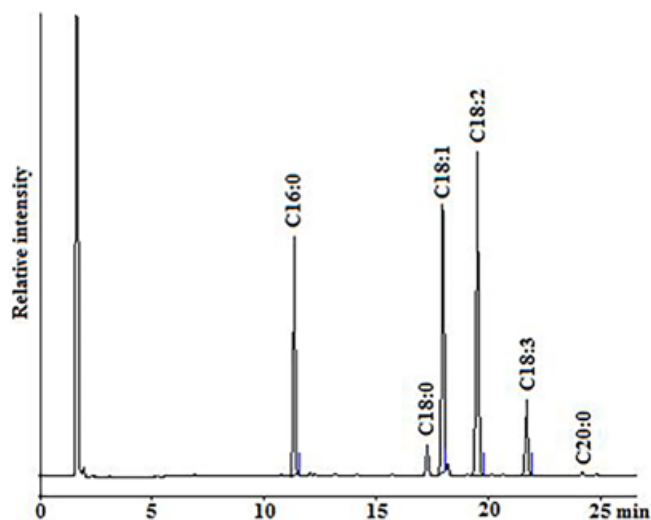
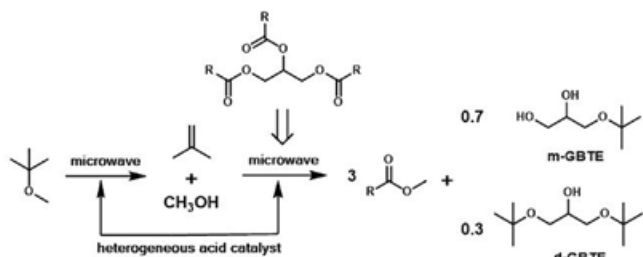


Figure 4. GC profile of fatty acid methyl ester production by enzymatic transformation.

for other applications in the nutraceutical or pharmaceutical fields.

FAME production by microwave-assisted heterogeneous catalytic transformation

In parallel to the biocatalyzed transesterification process, we developed an alternative microwave-assisted heterogeneous catalytic route for the production of biodiesel from lemon seed oil, applying a procedure developed in our laboratories some years ago: a *one-pot* microwave-assisted transformation of vegetable oils to a mixture of FAMES and glycerol ether derivatives, using commercially available MTBE.³⁶ This transformation was reported to be catalyzed by amorphous silica oxide functionalized with 10 wt% sulfonic groups. In this process, MTBE represents the single source both as



Scheme 1. Decomposition of methyl-*tert*-butyl ether, *trans*-esterification of triglycerides and glycerol etherification.

a transesterification and as a *trans*-etherification reagent, with the success of the process depending on the acidic catalyst's ability to perform simultaneously and effectively three distinct processes: the decomposition of MTBE; the transesterification of the triglycerides; and glycerol etherification (Scheme 1). The process was also found to be suitable for the transformation of acidic vegetable oil without any preliminary treatment. To optimize this reaction, we have investigated many kind of heterogeneous acid catalysts⁴⁵ and, according our results, the commercially available polystyrene sulfonic acid resins (beads) such as Amberlyst®-36 *dry form* are a very good compromise between efficiency and stability.

Therefore, we moved on to the investigation of the microwave-assisted heterogeneous catalytic transformation of two different kinds of oil extracted from *C. limon* seeds using analogous reaction conditions of the previously developed process, and Amberlyst®-36 *dry form* as the catalyst.

The mixture of each of the two oil samples and MTBE in the presence of a catalyst (1.5–2.0% w/w) was treated under microwave irradiation. The maximum temperature and pressure limits on the microwave reactor were set at 130°C and 20 bar, respectively, starting from a power of 20 W. The process quickly reached the limits of pressure and temperature thanks to the rapid decomposition of MTBE into isobutene and methanol, while the trend of the microwave power varied between 15 and 20 W along the entire process time. After 3 h, the complete conversion of triglycerides into a mixture of FAMEs and glycerol derivatives was observed (Fig. 5), mainly composed of *m*-GBTE [(±)-3-*tert*-butoxy-1,2-propanediol] and *d*-GBTE {1,3-bis[(2-methyl-2-propanyl)oxy]-2-propanol, ratio ~60:40}. No trace of glycerol was detected, as required by our primary objective. While the miscibility of oil and MTBE makes the reaction faster, the driving force behind the efficiency of the whole process is due to many interrelated factors that limit the reversibility of the entire process as we have previously described.³⁶

As for the modulation of the operating conditions, keeping the oil–MTBE ratio constant (almost 1:12 mol/mol), the quantity of catalyst of 2% (w/w) seems to be a reasonable

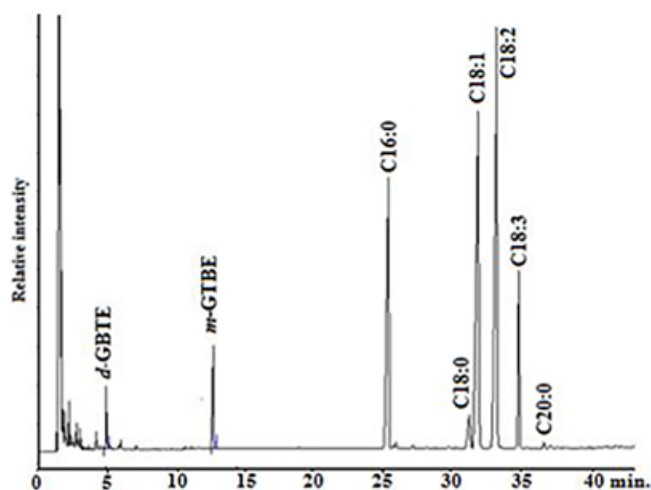


Figure 5. GC profile of fatty acid methyl ester production and glycerol ethers by microwave catalytic transformation.

compromise since, by reducing it, the reaction takes longer to complete, otherwise increasing it will not improve the process.

Finally, concerning the modulation of the microwave operating conditions, by reducing the pre-set maximum temperature, a decrease in internal pressure was observed, partly owing to the lower rate of isobutene formation, which slows down the entire transformation process. At a pre-set temperature below 100°C, after 3 h, we observed just a negligible triglyceride transformation to the corresponding FAMEs.

Conclusions

Herein, two different and efficient processes to convert oil extracted from *C. limon* seeds to a glycerol-free biodiesel have been reported. There are several reasons making these processes very attractive, in terms of both cost and sustainability.

Fatty acid methyl ester production by irreversible esterification catalyzed by CALB is a very convenient procedure, in fact, it is possible to use oils of different origins also with a high degree of acidity, as the mild reaction conditions allow transesterifications to be obtained without side-products, and above all for the simplicity of the process.

The commercially available polystyrene sulfonic acid catalyst, in combination with microwave radiation, is able to provide an effective transformation of the above-mentioned extracted oil to a mixture of FAMEs and glycerol ethers, without any pre-treatment. The obtained mixture, which is glycerol free, is separated from the solid catalyst through a very simple filtration. The excess of both TBME and

isobutene is simply recovered by a proper distillation, and then reused.

In conclusion, the absence of free glycerol in the final mixtures makes the two described heterogeneous catalytic processes remarkably advantageous in terms of both cost savings and sustainability since they allow the production of FAMES to be carried out without requiring steps to remove it. Both processes allow for easy recovery and the reuse of the catalyst itself, and the heterogeneity of both catalysts allows for easy reactor design and an affordable potential industrial process. In both examples, the final blends obtained are suitable for direct exploitation in the energy chain as a biofuel because they have the advantage of avoiding the post-treatment that is otherwise required on traditional plants for biodiesel production.

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Carmelo Drago

Carmelo Drago is a permanent researcher at the Institute of Biomolecular Chemistry (IBC-CNR) of Catania, where he is involved in research projects in the area of green chemistry concerning the development of both new heterogeneous catalysts and sustainable catalytic processes for the transformation of waste biomass into biofuels.



Raffaele Morrone

Raffaele Morrone is a researcher at the National Research Council. His research activity is focused on the area of biocatalysis in organic synthesis. In this context, he has prepared chiral organometallic compounds and enantiopure acids of pharmaceutical interest and has developed efficient biocatalytic methodologies.



Nicola D'Antona

Nicola D'Antona is a permanent researcher at the Institute of Biomolecular Chemistry and since 2016 Head of its Catania branch. His research activities are mainly focused on biocatalysis, green chemistry, stereochemistry and valorisation of waste biomasses.



Giuseppe Ruberto

Giuseppe Ruberto has been a Senior Researcher at the ICB-CNR since 1983. His research focuses on the chemistry of natural products taking care of isolation, structural determinations and biological activities, as well as their potential applications in the pharmaceutical and agro-alimentary fields. He is the leader of the Natural Products and Food Chemistry group of his Institute, and has authored about 200 publications.



Edoardo Napoli

Edoardo Napoli is a chemist and biotechnologist with a PhD achieved in 2003, and permanent research staff at ICB-CNR since 2008. His research activities are focused on natural product chemistry, phytochemistry, cosmetics, natural ingredients, flavors and fragrances and the exploitation of agroindustrial wastes.