Improvement of Biodiesel Production Based on the Application of Ultrasound: Monitoring of the Procedure by FTIR Spectroscopy

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ABSTRACT: A novel application of ultrasounds is presented for the improvement of the efficiency of the production of FAME (or biodiesel) from materials not used so far for this purpose, such as seed cakes. The novelty of this work is the introduction of *in situ* derivatization assisted by ultrasounds (ultrasonically assisted extraction transesterification) for biodiesel production. Thus, the TG contained in solid material are extracted and immediately transesterified in a methanolic solution of 1 M NaOH in an ultrasonic field. The total yield of FAME from seeds that contain TG is greatly increased in most instances. In the seeds used in this work, yields were increased from 46 to 85.5 % for cotton, 67.2 to 93 % for sunflower, and 43.2 to 83.5 % for sesame. An FTIR methodology was developed to determine the percentage of FAME in the *n*-hexane layer of the reaction and thus, to monitor the reaction process. Overall advantages of the proposed methodology include the elimination of saponification, low reaction time, milder reaction conditions, and higher FAME yields.

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KEY WORDS: Alkali-catalyzed transesterification, biodiesel production monitoring, FAME, FTIR spectroscopy, *in situ* transesterification, seed cakes, simultaneous extraction transesterification, TG, ultrasounds.

Biodiesel has been defined as the FA methyl esters (FAME) or FA ethyl esters derived from vegetable oils or animal fats (TG) by transesterification with methanol or ethanol. Its main advantages over fossil fuel are that it is renewable, biodegradable, and nontoxic. Its contribution to greenhouse gases is minimal, since the emitted CO_2 is equal to the CO_2 absorbed by the plants to create the TG (1,2).

Many countries have issued standards and regulations (Deutches Institut für Normung, DIN; Österreichisches Normungsinstitut, ÖNORM) describing the composition and physical properties of biodiesel (3). Attention is drawn to the maximum allowable content of saturated FAME, which depends on the plant from which the TG are derived and on the transesterification reaction conditions (4). The composition of plant-derived TG varies from one species to another. Therefore, plants that contain TG composed of out-of-standards FA can be used for biodiesel production provided their oil is mixed with that of others. Also, the reaction and storage conditions must be controlled to avoid oxidation and isomerization of the FAME double bonds (5).

The two most common ways to remove TG from plant materials are organic solvent extraction (*n*-hexane, isohexane) and mechanical pressure (oil expellers). The latter process removes a large percentage of existing TG, but some are left in the remaining cake. The transesterification reaction may be performed through several methods such as acidic, alkali, or enzyme catalysis (6,7), the first two taking place at higher temperatures than the last one. Zeolite and metal catalysis also have been proposed (8). All of the proposed methods have disadvantages such as long reaction times, formation of soap, and a high cost of consumables or infrastructure. The *in situ* production of FAME with the use of supercritical fluids or microwaves also has been suggested as a way to bypass the separate TG extraction step (9,10).

The application of low-frequency ultrasound recently has been suggested for fast, cost-efficient alkali-catalyzed transesterification of TG to FAME. This method also requires less catalyst, and it uses a more efficient molar ratio of methanol/TG, down to 6:1. Issues may arise regarding the separation of biodiesel from the leftover methanol, catalyst, and glycerol (11–13).

The percentage of TG that seed cakes contain varies depending on the applied pressure as well as on the physical and mechanical properties of the seeds. Increased pressure results in a temperature increase that may affect the TG composition of the product. Not all TG can be removed by mechanical pressure; the cakes remaining can only be used only as feedstock.

A new way to perform *in situ* reactions on TG-containing materials has been suggested. The application of ultrasound is known, and this work further supports its use, to aid the extraction and the transesterification reaction. The use of alkali-catalyzed ultrasonically assisted extraction transesterification (USAET) is proposed to facilitate the use of seed cakes for biodiesel production and to preserve some of the nutritional value of the cake, which might yet be used as animal feed.

An FTIR method was developed to monitor the various stages of USEAT. It also allows one to determine which comes first: the transesterification reaction in the TG-containing material or the extraction step. Unlike other methods for the analysis of TG and FAME (14), which are not as simple, rapid and nondestructive, FTIR is used to determine the degree of con-

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TABLE 1
Extraction and USAET Yields of Various Samples[a]

Sample	Method	Soxhlet (TG%)	USE (TG%)	USAET (FAME g/10 g sample)	USAET FAME/total TG content (%)
Cynara	seeds	24.2	23.4	1.9	78.5
Cotton	seeds	17.5	16.1	0.7	40
	cake	9.1	8.6	0.7	76.9
Sunflower	seeds	41.2	38.7	1.8	43.7
	cake	12.7	11.4	1.1	86.6
Sesame	seeds	58.3	55.7	3.6	61.7
	cakes	32.4	31.1	2.5	77.2

[a] USAET, ultrasonically assisted extraction transesterification; USE, ultrasonically assisted extraction.

version of TG to FAME. Other FTIR-based methods previously reported have used fiber optic probes (15) and attenuated total reflectance FTIR (16) for reaction monitoring. The mid-IR spectra were recorded using ZnSe windows against a ZnSe background, and spectral manipulation was carried out by specialized software.

EXPERIMENTAL PROCEDURES

Materials. *Cynara cardulus* seeds were kindly provided by the Laboratory of Crop Science (Agricultural University of Athens, Athens, Greece). Until use, the seeds were stored in a dark, dry room. Prior to use the seeds were milled in a cuisine blender. Seeds and seed cakes (cotton, sunflower, sesame) were obtained from a local oil producer in northern Greece once their oil had been mechanically removed. Cotton, sunflower, and sesame seeds are known for their oil content, and cynara seed is a promising new object for biodiesel production. The cakes were used as received for the extraction of TG. Solvents used were residue-level methanol (99.9%), NaOH pellets, and *n*-hexane (97%) purchased from BDH (Poole, England).

Isolation of TG from seeds and cakes. Soxhlet extraction was performed for each sample (10 g) as a reference method, with 300 mL of *n*-hexane for 4 h. Anhydrous $MgSO_4$ was then added to remove traces of water, and the solvent was removed with a rotary evaporator. The oil was then weighed on an analytical balance. The percent concentration of oil for each sample was then determined (Table 1).

All ultrasonic treatments were performed in an ultrasonic water bath operating at 35 kHz. Ten (10) grams of sample (seeds or cakes) was transferred to an Erlenmeyer flask with 50 mL of solvent (*n*-hexane). Extraction was performed for 10 min at 25°C. Two more consecutive extractions under the same conditions were performed to achieve 98% recovery of the total amount of oil, as was determined by the reference method.

Conversion of TG to FAME. USAET was performed for each sample (10 g of seeds and cakes with 50 mL of *n*-hexane and 3 mL of 1 M NaOH in methanol) in an ultrasonic water bath for 30 min at room temperature. The TG from cynara were transesterified to FAME with the application of ultrasound to either of two oil/solvent ratios, 1:1 and 1:25, with the same amount of catalyst as in the USAET with 1 g of oil. In all experiments, the methanol and hexane layers were separated using a separatory funnel. The hexane layer was dried with anhydrous $MgSO_4$, and the solvent was evaporated in a rotary evaporator. The remaining FAME was weighed on an analytical balance. TLC was used to determine the conversion of TG to FAME, together with the FTIR method described below. The conversion of pure oil was performed as reported previously (13).

TLC. TLC was performed on 0.2 mm silica gel plates (Merck, Darmstadt, Germany). The eluent solvents were chloroform/petroleum ether, 1:3 vol/vol. Spots were visualized by spraying with an ethanolic solution of phosphomolybdic acid (7.5% wt/vol in EtOH) and charring on a hot plate.

FTIR for process monitoring. TQ Analyst software (Thermo Electron Corp., Runcorn, United Kingdom) was used to develop a monitoring method for the quantitative simultaneous

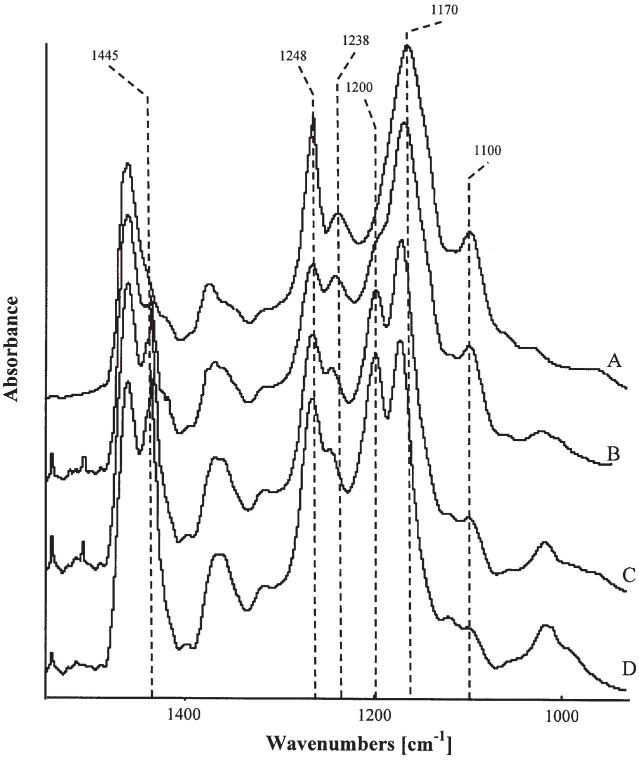

FIG. 1. FTIR spectral region 1500 to 900 cm^{-1} of TG (A) and FAME (D) and their 70:30 TG/FAME (B) and 30:70 TG/FAME (C) mixtures.

TABLE 2
Characteristic Absorption Frequencies and Assignments of the Spectral Region (1500–1060 cm^{-1}) Used for the Determination of FAME in TG/FAME Mixtures

Band position (cm^{-1})	Assignment	TG	FAME
1445	CH_3– asym. bend	—	+
1238–1248	O–H deformation	+	+
1200	O–CH_3 stretch	—	+
1170	C–O–C sym. stretch, C–C stretch	+	+
1100	O–CH_2–C asym., –CH_2–OH	+	—

determination of FAME and TG in their mixtures. By this means, it is possible to determine the conversion of TG to FAME instrumentally. TG oil and FAME were mixed in known concentrations to provide input spectra for the TQ Analyst reference. The software uses the partial least squares algorithm, with a constant path length, and uses the 1300 to 1060 cm^{-1} spectral region to determine the percentage of FAME in the TG/FAME mixture. All spectra were automatically smoothed and baseline-corrected prior to treatment, while the mean centering technique was used to scale the absorbance axis automatically. Spectra of TG, FAME, and their mixture are shown in Figure 1. The spectral region comprises a number of peaks assigned to various vibrations as shown in Table 2 (17,18).

RESULTS AND DISCUSSION

The objectives of this work were to improve TG extraction, primarily from cakes and secondarily from seeds, as well as to optimize the conversion of TG to FAME. Both were achieved by the application of low-frequency ultrasounds (Table 1).

The experiments carried out for both seeds and cakes samples show that only a fraction of the original TG content of the seed, as determined by Soxhlet extraction, is removed by mechanical pressure. The amount of oil remaining in the cake is 52% for cotton, 30.8% for sunflower, and 55.5% for sesame. This variation may be caused by the texture of the seeds.

Ultrasonically assisted extraction of TG from our samples proved to be as efficient as Soxhlet extraction, reaching about 95% recovery of TG. At the same time it consumed less time and solvent and subjected the sample to less thermal stress. Thus, the use of ultrasonic treatment in the extraction process is the first principle on which the USAET procedure will be based, since the extracted TG will be the reactants for the transesterification reaction that will take place at the same time. This critical point to the USAET concept is also represented (Table 1) by the USAET yield, measured as FAME, as a proportion of the total TG content in the sample.

The second principle is the application of ultrasound in the transesterification procedure. This recently developed method presents advantages such as short reaction times, efficient molar ratio of methanol to TG, and simplicity. However, some complications may arise in separating FAME from the glycerol, the other reaction product, and from the remaining methanol and catalyst when pure oil is transesterified (11).

This difficulty may be overcome by using a solvent to conduct the two-phase reaction. Thus, FAME are dissolved in the upper *n*-hexane layer, while all other products and residues remain dissolved in the glycerol layer. Equal masses of TG (1 g) were dissolved in different volumes of solvent (1 and 25 mL) to determine whether the concentration of reactants affected the reaction time and yields. The reaction time in both experiments was comparable with those previously reported (11), but no soap formation was observed, and the separation was easily held in a separatory funnel. The experimental procedure was monitored by TLC and FTIR. The reaction yield was close to 96%, and no admixture of glycerol in the *n*-hexane layer was detected in the –OH region of the FTIR spectra.

The absence of glycerol was also observed in the IR region used for the quantitative determination of FAME. The peak attributed to the C–CH_2–O vibration (1100 cm^{-1}) is reduced in the FAME spectra but it is present in the TG spectra. The region from 1300 to 1060 cm^{-1} includes absorption bands related directly to the presence of esters and, in our case, discriminates between glycerol esters and methyl esters mainly through the absorptions due to C–O vibrations. The most characteristic peak is that of the O–CH_3 (1200 cm^{-1}) initial methyl group that is added from the methesterification. The peak increases when the percentage of FAME is increased. Outside the region used for the quantitative determination, there is a very characteristic peak (1445 cm^{-1}) that is not quantitative, although it indicates the presence of the CH_3 group in mixtures of FAME.

The FTIR spectra recorded during the experiment can be used to determine the percentage of FAME quantitatively in the *n*-hexane layer. The data on percent FAME with time were used to construct logarithmic curves A and B in Figure 2 so as to represent the reaction process over the first 30 min. It can be seen that the reaction time for lower concentrations of cynara TG/*n*-hexane (1:25, line B) is the same as for higher concentrations (1:1, line A). This finding shows that the USAET procedure is advantageous when low concentrations of TG are

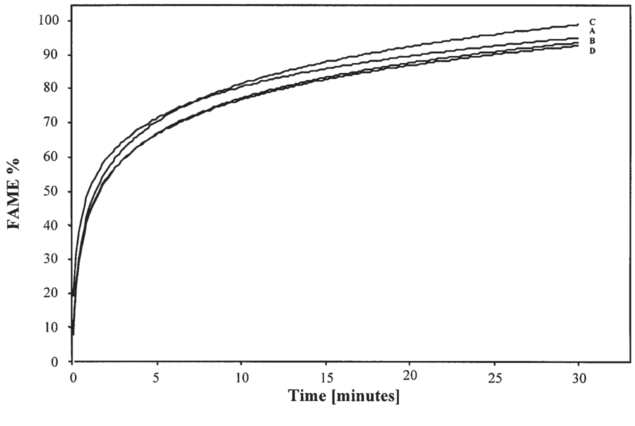

FIG. 2. Reaction curves for the monitoring of cynara TG conversion to FAME. (A) 1:1 oil/solvent, (B) 1:25 oil/solvent, and ultrasonically assisted extraction transesterification for sesame cake (C) and seeds (D).

transesterified in the process of their extraction from the sample (cakes, seeds).

In determining the optimal amount of methanol and catalyst, the USAET procedure was initially performed using 1 mL of 1 M MeOH/NaOH; in this instance, complete transesterification was not achieved, as indicated by TLC. The experiment was repeated several times, increasing the amount of catalyst by 0.5 mL of 1 M MeOH/NaOH at each step and performing the procedure with 10 g of sample. Thus, the minimum amount of catalyst needed to complete the USAET procedure was determined to be 3 mL. Molar ratios of 6:1 MeOH/TG, in the presence of 0.5–1.5% NaOH w/w of oil, previously have been reported for transesterification of pure TG oil to FAME (11). The amount of MeOH for the USAET procedure greatly exceeds the above levels, owing to the presence in the flask of a solid sample that absorbs some of the methanol, thus reducing the reaction efficiency. The increase in the amount of methanol does not necessarily mean that it is spent, as it can be easily recycled and at the same time it dissolves the glycerol. Regarding the high concentration of NaOH in methanol, it is assumed that the presence of various acids in the seeds or cakes will neutralize some of the catalyst. Also, the water present in the samples hydrolyzes some of the catalyst (the methoxy anion CH_3O^-). Finally the high concentration of catalyst will result in uncontrolled side reactions if the temperature is higher than 30°C (11,19).

The optimal conditions were applied in the USAET procedure for all samples. The USAET reaction, as monitored by FTIR, is shown in Figure 2 for sesame cake and seeds (lines C and D, respectively). Although the USAET procedure has been developed primarily to allow the use of cakes as a new source of biodiesel, it can also be successfully applied in the case of seed samples. The main objective was achieved, as the proportion of the transesterified TG from cakes with the USAET procedure was within 76.7 to 86.6% of the TG originally contained in the cakes (Table 1). The lack of mechanical treatment of the seed samples reduced the efficiency of the USAET procedure to 40–61.7% (Table 1).

For cotton seeds, 40% of the total TG originally contained in the seed was transesterified with the USAET procedure. If the standard method of mechanical removal of the TG from the seed with subsequent conversion to FAME were applied, 48% of the total TG would be removed and then converted at best by 96% according to our data. Hence, the total FAME derived by the standard method from cotton would be 46% which is comparable to the 40% direct conversion of USAET.

Similarly, for sunflower seeds, 70% of the TG is mechanically removed. If they are then converted to FAME 96%, the total FAME yield would be 67.2%, which is higher than the 43.7% of USAET. For sesame seeds, 45% of TG is mechanically removed, giving, at a 96% conversion rate, a total FAME yield of 43.2%, which is very low compared with the 61.7% of USAET. It can be assumed that the texture, size, and hardness of each individual seed affect their behavior as potential biodiesel sources.

More impressive results are observed if the USAET procedure is applied to cakes obtained from seeds after mechanical removal of some of the TG. For cotton, as shown above, 46% of their TG are converted to FAME by the standard procedure. The TG from cakes (52% of the amount initially contained in the seed) are 76.9% transesterified by using the USAET (Table 1). Thus, an addition 39.5% of the initial TG (which is 76.9% of the 52% TG from the seed) is converted to biodiesel, thus increasing the overall yield of FAME from 46 to 85.5%.

Accordingly, the application of USAET improved transesterification of TG from 67.2 to 93% for sunflower oil and from 43.2 to 83.5% for sesame oil.

The FTIR data also provide a deeper insight into the exact sequence of the extraction and reaction steps. The presence of TG in the hexane layer during the USAET procedure, as shown by FTIR, suggests that the extraction step precedes the reaction step by a short but measurable amount of time. However, a small degree of transesterification may actually occur in the plant tissue.

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