

## Improving biodiesel yield from pre-esterified inedible olive oil using microwave-assisted transesterification method

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**SUMMARY:** In the present research, biodiesel production from olive oils with different initial free fatty acid concentrations (2.5, 5.0, and 10.0%) was evaluated. A two-stage acid-catalyzed esterification and alkaline-catalyzed transesterification (ACT) process using the microwave heating method was compared with the traditional heating method. Free fatty acid was reduced to less than 2.0% in the first stage. Although no significant difference was observed between microwave and traditional esterification methods in terms of fatty acid reduction, the microwave treatment significantly decreased reaction time by 92.5%. Comparing microwave ACT results with those of the traditional heating method showed that the microwave can significantly increase methyl ester yield and purity, and simultaneously decrease reaction time. Physical constants of methyl esters were also improved using the microwave heating method. Therefore, the microwave heating method can be regarded as an efficient method instead of the two-stage method for biodiesel production. This method is capable of using inedible olive oil with high concentrations of free fatty acids.

**KEYWORDS:** *Biodiesel; Esterification; Microwave; Olive oil; Transesterification*

**RESUMEN:** *Mejora del rendimiento del biodiesel a partir de aceite de oliva no comestible pre-esterificado mediante transesterificación asistida por microondas.* En la presente investigación, se evaluó la producción de biodiesel a partir de aceites de oliva con diferentes concentraciones iniciales de ácidos grasos libres (2,5, 5,0 y 10,0%). Se comparó un proceso de esterificación en dos etapas catalizada con ácido y transesterificación catalizada alcalina (ACT) usando microondas con el método de calentamiento tradicional. Los ácidos grasos libres se redujeron a menos del 2,0% en la primera etapa. Aunque no se observaron diferencias significativas entre los métodos de esterificación, por microondas y tradicional, en términos de reducción de ácidos grasos, sin embargo, el microondas disminuyó significativamente el tiempo de reacción en un 92,5%. La comparación de los resultados de ACT de microondas con los del método de calentamiento tradicional mostró que el microondas puede aumentar significativamente el rendimiento y la pureza del éster metílico, y simultáneamente disminuir el tiempo de reacción. Las constantes físicas de los ésteres metílicos también se mejoraron usando el método de calentamiento por microondas. Por lo tanto, el método de calentamiento por microondas puede considerarse como un método eficiente en lugar de la producción de biodiésel en dos etapas. Este método es capaz de usar aceite de oliva no comestible con altas concentraciones de ácidos grasos libres.

**PALABRAS CLAVE:** *Aceite de oliva; Biodiesel; Esterificación; Microonda; Transesterificación*

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## 1. INTRODUCTION

Biodiesel or fatty acid alkyl ester is produced via transesterification, which is the reaction of triacylglycerol with an alcohol using an appropriate catalyst. Normally, transesterification is an alkaline-catalyzed reaction which is 4000 times faster than an acid-catalyzed reaction (Fukuda *et al.*, 2001). The cost of biodiesel production is one of the main constituents which can be decreased by choosing waste oils as the source of feedstock (Jaliliannosrati *et al.*, 2013). Meher *et al.* (2006) investigated the alkaline-catalyzed transesterification of karanja (*Pongamia pinnata*) oil. They showed that biodiesel yield decreased from 96 to 6.1% by increasing the free fatty acid (FFA) concentration from 0.48 to 5.75%. Therefore, in order to complete the alkaline-catalyzed reaction, a FFA value of lower than 3% is needed. The high amounts of FFAs in biodiesel feedstocks make them unsuitable for alkaline-catalyzed transesterification due to the soap formation between FFAs and the alkaline catalyst (Vicente *et al.*, 2004). Soap decreases the reaction yield and acts as a surfactant between the two final immiscible products (methyl ester and glycerol), making downstream separation more difficult and leading to an increase in biodiesel viscosity and purification costs (Mazubert *et al.*, 2014; Sarantopoulos *et al.*, 2014). A similar explanation has been reported in the study by Thoai *et al.* (2017) on the transesterification of refined palm oil. They showed that by increasing soap concentration from 1.48 to 3.21%, biodiesel purity decreased from 98 to 85%. In addition to soap, water is also another product of saponification. When water is present in the reaction, it generally manifests itself through excessive soap production. Park *et al.* (2016) investigated the transesterification of wet coffee grounds. They noted that by increasing water from 20 to 80%, biodiesel yield decreased from about 98 to less than 40%. Also, soaps of saturated fatty acids tend to solidify at ambient temperatures. Consequently, a reaction mixture with excessive soap may gel and form a semi-solid mass which is very difficult to recover (Van Gerpen *et al.*, 2004).

Olive oil (OO) has been playing an important role in the world market of vegetable oils. The annual worldwide production of virgin OO was 3,050,390 tonnes in 2014, with Spain as its main producer (57% of the global production; 1,738,600 tonnes/year) (FAOSTAT, 2014). In fact, in Spain,

edible vegetable oil consumption is approximately 600,000 tonnes/year. Most of this oil (70%) is OO and is primarily used for deep frying. According to the INE (Spanish National Institute of Statistics), about 74,000 tonnes of waste OO is collected per year. In this sense, the transesterification of waste OO for producing biodiesel could decrease the waste disposal problem. Although several approaches have been developed to produce biodiesel from vegetable oils and animal fats, research concerning biodiesel production from waste OO is limited and further research is needed. Due to the presence of moisture and hydrolytic enzymes, the FFA concentration in the waste OO rises rapidly (Shahidi, 2005). Dorado *et al.* (2003) and Yuste and Dorado (2006) investigated the transesterification of waste OO for biodiesel production using a conventional heating method. Waste OO with high FFA is inedible and its refining is costly. Therefore, this high free fatty acid olive oil (HFFA OO) is economically suitable for biodiesel production. Due to undesirable outcomes, HFFA OO needs to be pre-treated before transesterification. Kara *et al.* (2017) applied an acid-catalyzed esterification pre-treatment for reducing high FFA concentrations in waste fish oil from 28 to less than 1.5%. The pre-treated oil is then transformed using alkaline-catalyzed transesterification (Sarantopoulos *et al.*, 2014).

Several heating methods have been investigated for biodiesel production. In conventional heating methods, heat energy is transferred to the raw material by convection and conduction from the hot surface of the reactor, which requires a long time and large amounts of energy (Talebian-Kiakalaieh *et al.*, 2013). Microwave radiation is a novel heating method which can be used for a large number of chemical reactions such as esterification and transesterification (Talebian-Kiakalaieh *et al.*, 2013). Generally, two mechanisms of dipolar rotation and ionic conduction have been introduced for the interaction between microwave energy and raw materials. Polar materials such as methanol can absorb microwave radiation and the ionic molecules, such as KOH, can be divided into positive and negative ions. At the same time, the probability of molecular encounters increases by accelerating the molecular/ionic movement which leads to an increased reaction rate (Sajjadi *et al.*, 2014). Lin and Chen (2017) transesterified *Jatropha* oil and showed that for obtaining biodiesel purity

higher than 90%, microwave requires just 10 seconds while the conventional hot plate requires 1 hour. Also, energy consumptions of conventional hot plate and microwave heating methods were 2376 and 9.5 kJ, respectively.

Even though some studies have proposed a two-step reaction of acid-catalyzed esterification followed by alkaline-catalyzed transesterification for biodiesel production, none of them is based on comparison between different heating methods in both steps. Therefore, the aim of the present study was to use a microwave heating method for esterification of OO with different initial FFA concentrations followed by its transesterification and to compare it with the conventional magnetic stirrer method. Purity, yield, physical properties, and composition of the fatty acid methyl esters (FAMES) produced were also compared.

## 2. MATERIALS AND METHODS

### 2.1. Materials

All experimental chemicals (solvents, reagents, and standards) were of analytical grade and were purchased from Merck (Darmstadt, Germany) and Sigma–Aldrich (St. Louis, MO). Crude OO mill (three-phase centrifuge) was supplied from Etko Oil Company (Rudbar, Iran).

### 2.2. Methods

#### 2.2.1. Acid-catalyzed esterification

Microwave-assisted esterification (MAE) was carried out with sulfuric acid (10%, w/w H<sub>2</sub>SO<sub>4</sub>/FFA) as catalyst. The catalyst was dissolved in methanol (methanol to FFA mole ratio of 40) and was stirred for 5 min to ensure complete mixing. According to the American Oil Chemists' Society (AOCS) Official Method (Ca 5a-40), FFA of OO was 2.36% which increased to 2.5, 5.0, and 10.0% by pure oleic acid (AOCS, 2000). The catalyst–methanol solution was mixed with 100 g high FFA OO. The reaction was carried out for 9 min in a domestic microwave oven (ME3410W, Samsung Malaysia Electronics, Kuala Lumpur, Malaysia) at 500 W with a wave frequency of 2450 MHz, equipped with a condenser (to condense back the methanol escaping from the reaction mixture). Also, the magnetic stirrer operated at 60 °C for 120 min at 600 rpm as a conventional-assisted esterification

(CAE). At the end of the reaction time, the reaction mixture was immediately cooled down to room temperature. The water-soluble components were separated from the product in a separatory funnel. The esterification yield was calculated according to the following equation (Chai *et al.*, 2014):

$$\text{Esterification yield (\%)} = ((\text{initial FFA} - \text{final FFA}) / \text{initial FFA}) \times 100 \quad \text{eq. (1)}$$

#### 2.2.2. Alkaline-catalyzed transesterification

KOH was dissolved in methanol and the mixture was stirred for 10 min to ensure complete mixing. Then, the catalyst-methanol solution was mixed with 100 g esterified HFFA OO. The reaction was carried out using the microwave oven and the conventional magnetic stirrer.

Microwave-assisted transesterification (MAT) of esterified HFFA OO was carried out at 500 W, methanol-to-oil mole ratio of 9, KOH concentration of 1.2%, and reaction time of 9 min. Conventional-assisted transesterification (CAT) was performed using a magnetic stirrer at 60 °C for 120 min at 600 rpm. To evaluate the effect of esterification pre-treatment on transesterification yield, OO samples (with 2.5, 5.0, and 10.0% FFA) were also transesterified without esterification pre-treatment.

At the end of the reaction time, the reaction mixture was immediately cooled down to room temperature and transferred to a separating funnel where it was left overnight to separate into two phases. The crude methyl ester remained in the upper phase, while the catalyst and unreacted methanol were situated in the lower glycerol phase, meaning that small amounts of catalyst, methanol, and glycerol were present in the upper phase. The excess methanol in the ester phase was distilled off with a magnetic stirrer equipped with a condenser at 70 °C for 30 min at 450 rpm. Hot distilled water (60 °C) was sprayed over the surface of ester phase to remove the impurities and catalyst. Washing was performed 5 times to remove all dissolved catalysts and glycerol in the ester phase. The lower phase was discarded and a yellow-colored phase containing FAMES was finally isolated. FAMES were then dried using a magnetic stirrer at 80 °C for 30 min at 250 rpm (Kanitkar *et al.*, 2011; Patil *et al.*, 2011). Margaric acid methyl ester (Methyl margarate; Methyl heptadecanoate) was added to the crude

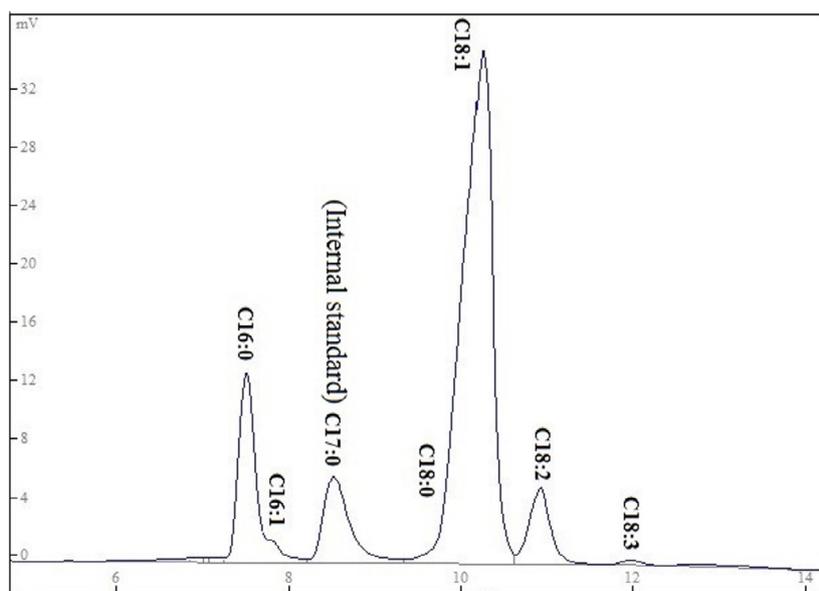


FIGURE 1. Chromatogram of microwave-assisted transesterified inedible olive oil.

FAMES as an internal standard (Figure 1) (Atapour and Kariminia, 2011). The purity of FAMES was determined using the GC/FID method according to the method described by Golmakani *et al.*, (2012a; 2012b). The weight of yield, methyl ester purity, and final yield were calculated according to the following equations (Patil *et al.*, 2011; Hsiao *et al.*, 2010):

$$\text{Weight of yield (\%)} = \left( \frac{\text{Weight of final product (g)}}{\text{Weight of olive oil (g)}} \right) \times 100 \quad \text{eq. (2)}$$

$$\text{Methyl ester purity (\%)} = \left( \frac{\text{Area of final product}}{\text{Area of internal standard}} \right) \times \left( \frac{\text{Weight of internal standard (g)}}{\text{Weight of final product (g)}} \right) \times 100 \quad \text{eq. (3)}$$

$$\text{Final yield (\%)} = \left( \frac{\text{Methyl ester purity (\%)} \times \text{Weight of yield (\%)}}{100} \right) \quad \text{eq. (4)}$$

### 2.2.3. Physical properties of FAME

Kinematic viscosity, refractive index, and density of purified FAMES were analyzed according to the method of the American Society for Testing Materials (ASTM; D445), AOCS Cc7-25 Official Method, and AOCS 1a-64 Official Method, respectively (AOCS, 2000; ASTM, 2013). The fatty acid composition of FAMES was determined according to the method described by Golmakani *et al.*, (2012a; 2012b). Color attributes ( $L^*$ , lightness;  $a^*$ , greenness to redness;  $b^*$ , blueness to yellowness) of FAMES were analyzed using the Habibi *et al.* (2016) method.

### 2.2.4. Statistical analysis

All experiments were performed in triplicate and the data were reported as mean values of the measurements. Standard deviation values are presented in all tables. A general linear model (GLM) procedure from SAS (Statistical Analysis Software, version 9.1; SAS Institute Inc. Cary, NC) was used for comparison of mean values.

## 3. RESULTS

### 3.1. Esterification yield

The effects of initial FFA concentration and esterification method on the esterification yield of HFFAEO are shown in Table 1. The highest esterification yield was obtained for 2.5% initial FFA (i.e. final FFA of lower than 0.5%). Although there were no significant differences between the esterification yield of HFFAEO with 5.0% FFA and that of 10.0%, their esterification yields were lower than that of the sample with 2.5% FFA. Also, despite the fact that there were no significant differences between MAE and CAE methods in terms of final FFA concentration and esterification yield, MAE entailed significantly lower esterification duration and energy consumption. Microwaves heat methanol selectively which may lead to the rapid formation of microzones (hot spots) with temperatures much higher than that of

TABLE 1. Effect of microwave heating method on esterification yield of high free fatty acid olive oil

Initial free fatty acid concentration	Esterification method	Final free fatty acid concentration	Esterification yield (%)
2.5	Conventional	0.47±0.02c*	81.44±0.79a
2.5	Microwave	0.45±0.01c	82.00±0.36a
5	Conventional	1.01±0.01b	80.10±0.03b
5	Microwave	1.01±0.01b	80.08±0.06b
10	Conventional	2.06±0.08a	79.48±0.50b
10	Microwave	1.98±0.04a	80.27±0.38b

\* Mean ± standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance:  $P < 0.05$ ; In each column, means with different letters are significantly different.

TABLE 2. Effect of different esterification and transesterification methods on weight of yield, purity, and final yield of olive oil biodiesel

Initial free fatty acid concentration	Esterification method	Transesterification method	Weight of yield (%)	Purity (%)	Final yield (%)
2.5	WEP*	Conventional	82.36±0.03m**	96.03±0.03d	79.09±0.06i
2.5	WEP	Microwave	85.72±0.01l	97.86±0.18c	83.88±0.17f
2.5	Conventional	Conventional	93.15±0.01g	89.33±0.76e	83.21±0.71g
2.5	Conventional	Microwave	98.57±0.05b	99.94±0.03a	98.50±0.08a
2.5	Microwave	Conventional	93.36±0.01f	89.77±0.25e	83.81±0.22fg
2.5	Microwave	Microwave	98.98±0.03a	99.94±0.03a	98.93±0.00a
5	WEP	Conventional	68.32±0.03o	2.92±0.07i	2.00±0.05k
5	WEP	Microwave	76.72±0.01n	3.18±0.08i	2.44±0.06k
5	Conventional	Conventional	92.08±0.04i	87.90±1.08fg	80.94±0.96h
5	Conventional	Microwave	96.51±0.01d	99.82±0.03a	96.33±0.03c
5	Microwave	Conventional	92.27±0.03h	88.24±0.24f	81.42±0.24h
5	Microwave	Microwave	97.60±0.02c	99.90±0.01a	97.50±0.03b
10	WEP	Conventional	44.06±0.01q	0.65±0.02j	0.28±0.01l
10	WEP	Microwave	52.33±0.01p	0.68±0.02j	0.36±0.01l
10	Conventional	Conventional	89.08±0.05k	87.07±0.05h	77.55±0.00j
10	Conventional	Microwave	95.37±0.01e	98.62±0.13b	94.05±0.14e
10	Microwave	Conventional	90.13±0.03j	87.24±0.13hg	78.63±0.15i
10	Microwave	Microwave	96.46±0.04d	98.90±0.01b	95.40±0.05d

\* WEP: Without Esterification pre-treatment; \*\* Mean ± standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance:  $P < 0.05$ ; In each column, means with different letters are significantly different.

the reaction mixture, thus providing enough energy for increasing the esterification rate (Sajjadi *et al.*, 2014). Unlike microwave, the conventional heating method heats the entire reaction mixture (both methanol and OO), leading to longer reaction time and higher energy consumption (Kumar *et al.*, 2011). These findings are in good agreement with the results of Suppalakpanya *et al.* (2010), who produced ethyl ester from crude

palm oil. They showed that MAE and CAE methods reduced FFA concentration from 7.5 to less than 2% after reaction times of 60 and 240 min, respectively.

### 3.2. Transesterification yield

The effects of different initial FFA concentrations and transesterification methods on weight of yield, purity, and final yield of HFFAOO are shown in

Table 2. The weight of yield, purity, and final yield of transesterified HFFAOOs without esterification were significantly lower than those of esterified ones. The final yield of HFFAOOs with 2.5, 5.0, and 10.0% FFA were 81.48, 2.22, and 0.32%, respectively. There were no significant differences between the final yield of OO with 5.0 and 10.0% FFA. In view of the fact that high initial FFA concentration increases the saponification reaction, esterification pre-treatment plays a key role in increasing transesterification purity and yield. MAT significantly increased the final yield of OO with 2.5% FFA in comparison with that from the CAT method.

In the case of esterified samples (both CAT and MAT methods), the weight of yield, purity, and final yield of FAMES decreased by increasing the initial FFA concentration. Higher initial FFA concentration leads to increasing soap formation, which resulted in decreasing biodiesel purity and yield.

The highest final yield was obtained using the microwave heating method both in the esterification and transesterification steps. Also, the lowest final yield was obtained using the conventional heating method in both the esterification and transesterification steps. MAT showed higher weight of yield, purity, and final yield in comparison with the CAT method. Microwave radiation can selectively heat polar molecules (alcohols) without significantly heating non-polar molecules (triacylglycerols), which promotes transesterification rate and hence improves the yield and purity of FAMES. In the conventional heating method, energy is transferred to the reaction mixture through convection and conduction only, which requires a long time and large amounts of energy (Wahidin *et al.*, 2014). Similar to our results, Wahidin *et al.* (2014) investigated the effects of MAT on the biodiesel yield of microalgal *Nannochloropsis oculata* oil. They reported that the final yield of MAT (86.41%) was higher than that of the conventional water bath method (71.26%).

### 3.3. FAME analysis

#### 3.3.1. Fatty acid composition

The effect of different esterification and transesterification methods on the fatty acid composition of the biodiesel produced are shown in Table 3. There were no significant differences among the fatty acid compositions of different initial FFA concentrations or different esterification and transesterification methods.

Therefore, transesterification rate is not dependent on the chain length or saturation degree of fatty acids. This phenomenon can be related to the speed and completeness of the transesterification reaction. In contrast to our finding, Stavarache *et al.* (2007) noted that saturated fatty acids, which are commonly located in the 1 and 3 positions of triacylglycerols, had a higher transesterification rate.

#### 3.3.2. Physical properties of transesterified samples

The effect of different esterification and transesterification methods on the physical properties of the biodiesel produced are shown in Table 4. The kinematic viscosity of FAME is one of the most important properties which can affect storage condition, transportation, and biodiesel operation. HFFAOO samples transesterified without esterification had higher kinematic viscosity than those of esterified samples (both CAT and MAT methods). The kinematic viscosity of unesterified samples with 5.0 and 10.0% FFA after transesterification was similar to untreated OO (i.e. without esterification and transesterification; 36.806 mm<sup>2</sup>/s). This phenomenon can be related to the fact that although most triacylglycerols participated in the saponification reaction instead of transesterification, transesterification was not affected by saponification in the case of OO with 2.5% FFA. Also, in spite of the fact that the transesterification method had no significant effect on kinematic viscosity, the MAT of OO with 2.5% FFA had significantly higher kinematic viscosity than the CAT method.

According to ASTM D6751 Official Methods, the kinematic viscosity of FAMES should be 1.9-6 mm<sup>2</sup>/s. Except for the FAMES of OO with 5.0 and 10.0% FFA transesterified without esterification, all the FAMES fall within the standard range indicated by ASTM.

In the case of esterified samples, MAT samples had lower kinematic viscosity than those of the CAT method. By increasing the initial FFA concentration from 2.5 to 10.0%, the kinematic viscosity of FAMES increased due to soap formation. There was a significant negative correlation between kinematic viscosity and final yield ( $R^2 = 0.972$ ; kinematic viscosity =  $(-0.065 \times \text{Final yield}) + 10.413$ ). During the transesterification process, triacylglycerols are converted to lower molecular weight FAMES (Talebian-Kiakalaieh *et al.*, 2013).

TABLE 3. Effect of different esterification and transesterification methods on fatty acid composition of olive oil biodiesel

Initial free fatty acid concentration (%)	Esterification method	Transesterification method	Fatty acid (%)					
			Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	$\alpha$ -Linolenic acid
2.5	WEP*	Conventional	15.53±0.05**	1.30±0.05	2.19±0.85	71.78±0.85	8.95±0.00	0.25±0.01
2.5	WEP	Microwave	15.97±0.07	1.36±0.08	1.92±0.48	71.55±0.48	8.95±0.00	0.25±0.05
2.5	Conventional	Conventional	14.54±0.00	1.37±0.00	1.94±0.00	72.03±0.00	9.65±0.00	0.47±0.00
2.5	Conventional	Microwave	15.50±0.09	1.36±0.08	1.42±0.11	72.17±0.12	9.08±0.00	0.47±0.02
2.5	Microwave	Conventional	14.65±0.03	1.26±0.03	1.94±0.00	72.03±0.00	9.65±0.00	0.47±0.00
2.5	Microwave	Microwave	15.60±1.02	1.36±0.16	2.40±0.18	71.56±0.96	8.63±0.63	0.45±0.03
5	WEP	Conventional	15.15±0.79	1.50±0.00	2.50±0.00	71.10±0.04	9.35±3.83	0.40±0.00
5	WEP	Microwave	15.13±0.76	1.50±0.00	2.50±0.00	72.33±0.61	8.24±8.37	0.30±0.07
5	Conventional	Conventional	15.64±0.07	1.17±0.07	1.86±0.16	72.30±0.16	8.71±0.00	0.32±0.03
5	Conventional	Microwave	15.54±0.04	1.32±0.04	1.35±0.22	72.24±0.22	9.08±0.00	0.47±0.00
5	Microwave	Conventional	15.55±0.03	1.26±0.03	1.48±0.38	72.68±0.38	8.71±0.00	0.32±0.03
5	Microwave	Microwave	15.41±0.12	1.43±0.15	2.82±0.73	71.06±0.33	8.89±0.26	0.39±0.11
10	WEP	Conventional	15.37±0.36	1.50±0.00	1.95±0.00	71.56±1.54	9.32±2.90	0.30±0.04
10	WEP	Microwave	15.22±4.82	1.20±0.00	2.00±0.00	71.97±1.50	9.21±5.31	0.40±0.02
10	Conventional	Conventional	14.65±0.01	1.26±0.01	1.30±0.46	72.70±0.45	9.62±0.00	0.47±0.08
10	Conventional	Microwave	15.54±0.04	1.32±0.04	1.35±0.22	72.24±0.22	9.08±0.00	0.47±0.00
10	Microwave	Conventional	15.58±0.01	1.44±0.01	2.27±0.92	71.05±0.92	9.37±0.00	0.29±0.03
10	Microwave	Microwave	15.42±0.08	1.44±0.08	2.02±0.40	71.58±0.40	9.07±0.00	0.47±0.06

\* WEP: Without esterification pre-treatment; \*\* Mean ± standard deviation; Number of replicates for each analysis: 3.

TABLE 4. Effect of different esterification and transesterification methods on physical properties of olive oil biodiesel

Initial free fatty acid concentration (%)	Esterification method	Transesterification method	kinematic viscosity (mm <sup>2</sup> /s)	Density (Kg/m <sup>3</sup> )	Refractive index	Color attribute		
						L*	a*	b*
2.5	WEP**	Conventional	5.194±0.000c***	881.1685±0.0637g	1.4544±0.0001d	58.50±0.35d	-8.25±0.35cd	44.50±0.71c
2.5	WEP	Microwave	4.159±0.000f	885.1601±0.0920d	1.4531±0.0001de	60.75±0.35ab	-8.60±0.14cd	39.00±0.00de
2.5	Conventional	Conventional	5.126±0.000d	881.9488±0.0495f	1.4544±0.0001d	59.25±0.35cd	-8.60±0.14cd	43.50±0.71c
2.5	Conventional	Microwave	4.019±0.001hi	886.9658±0.0424a	1.4526±0.0001fg	61.50±0.71a	-9.25±0.35e	36.50±0.71f
2.5	Microwave	Conventional	5.018±0.001e	883.1693±0.0637e	1.4544±0.0001d	58.50±0.71d	-8.75±0.07de	42.90±0.14c
2.5	Microwave	Microwave	4.016±0.001i	886.9658±0.2404a	1.4524±0.0001g	61.50±0.71a	-9.25±0.35e	36.50±0.71f
5	WEP	Conventional	36.752±0.033b	874.5209±0.0141h	1.4629±0.0001b	56.50±0.71e	-6.65±0.21b	54.50±0.71b
5	WEP	Microwave	36.760±0.001b	874.8761±0.0212h	1.4627±0.0000b	56.55±0.07e	-6.75±0.07b	54.10±0.14b
5	Conventional	Conventional	5.127±0.000d	881.9288±0.1344g	1.4545±0.0001d	59.24±0.34cd	-8.60±0.14cd	43.50±0.71c
5	Conventional	Microwave	4.035±0.001h	887.0408±0.0212a	1.4525±0.0002g	61.50±0.71a	-9.25±0.35e	36.50±0.71f
5	Microwave	Conventional	5.118±0.000d	881.9338±0.1132f	1.4545±0.0003d	59.25±0.35cd	-8.60±0.14cd	43.50±0.71c
5	Microwave	Microwave	4.018±0.000j	887.0008±0.0071a	1.4526±0.0001fg	61.50±0.71a	-9.25±0.35e	36.50±0.71f
10	WEP	Conventional	36.798±0.000a	874.1603±0.0134i	1.4692±0.0001a	56.49±0.01e	-5.25±0.35a	56.25±0.35a
10	WEP	Microwave	36.798±0.000a	874.1898±0.0014i	1.4691±0.0001a	56.51±0.01e	-5.40±0.14a	55.75±0.35a
10	Conventional	Conventional	5.207±0.001c	881.1685±0.0637g	1.4552±0.0002c	58.50±0.71d	-8.10±0.14c	44.50±0.71c
10	Conventional	Microwave	4.106±0.000g	885.8954±0.3537c	1.4529±0.0001fe	61.05±0.07ab	-8.75±0.35de	37.90±0.14e
10	Microwave	Conventional	5.194±0.000c	881.1885±0.0637g	1.4553±0.0004c	58.50±0.71d	-8.10±0.14c	44.50±0.71c
10	Microwave	Microwave	4.104±0.000g	886.240±0.0071b	1.4529±0.0001fe	61.10±0.14ab	-8.75±0.35de	37.90±0.14e

\*\* WEP: Without esterification pre-treatment; \*\*\* Mean ± standard deviation; Number of replicates for each analysis: 3; Statistical test: ANOVA and multiple comparison of means using Duncan's test; Degree of significance: P < 0.05; In each column, means with different letters are significantly different.

Hence, by increasing the biodiesel final yield, kinematic viscosity decreased accordingly. The sample with the highest final yield (98.93%) showed the lowest kinematic viscosity (4.02 mm<sup>2</sup>/s). In both esterification and transesterification steps, the microwave heating method was more effective than the conventional heating method due to its higher yield of FAME production and accordingly lower kinematic viscosity.

For both CAT and MAT methods, HFFAOO samples transesterified without esterification had lower density in comparison with esterified samples. In the case of unesterified samples, although the transesterification method of OO with 5.0 and 10.0% FFA had no significant effect on density, microwave-assisted transesterified OO with 2.5% FFA had significantly higher density than the CAT method.

In the case of transesterified samples, the MAT of OO showed higher density than that of the CAT method. By increasing the initial FFA concentration, the densities of FAMEs were decreased. There was a significant positive correlation between density and final yield ( $R^2 = 0.98$ ; Density =  $(0.299 \times \text{Final yield}) + 857.720$ ). The lower molecular weight of FAMEs in comparison with their corresponding triacylglycerols plays a key role in determining the density of the product (Motasemi and Ani, 2012). Therefore, by increasing final yield (i.e. higher production of lower molecular weight FAMEs), the density of the produced biodiesel increased. Using MAT led to higher final yield and hence density.

The refractive indices of HFFAOO samples transesterified without esterification were higher than those of esterified samples using both CAE and MAE methods. Also, different transesterification methods had no significant effect on refractive indices of OO with 2.5, 5.0, or 10.0% FFA.

In the case of esterified HFFAOOs, microwave-assisted transesterified samples had lower refractive indices. By increasing initial FFA concentration, the refractive indices of FAMEs increased. There was a significant negative correlation between refractive index and final yield ( $R^2 = 0.982$ ; Refractive index =  $(-0.0001 \times \text{Final yield}) + 1.465$ ). By increasing final yield, refractive index decreased due to the conversion of triacylglycerols to lower molecular weight FAMEs. Also, microwave-assisted transesterified samples showed lower refractive indices according to their higher final yield.

Color is a qualitative parameter for evaluating the effects of the transesterification process on the biodiesel produced. HFFAOO samples transesterified without esterification had lower L\*, a\*, and b\* values than those of esterified samples using either conventional or microwave methods. Regarding unesterified samples, although different transesterification methods of OO with 5.0 and 10.0% FFA had no significant effect on L\*, a\*, and b\* values, microwave-assisted transesterified OO with 2.5% FFA had significantly higher L\* value and lower b\* value than those of the CAT method. Also, the a\* value for OO with 2.5% FFA was similar in both MAT and CAT methods.

In the case of esterified samples, microwave-assisted transesterified samples had higher L\* values and lower a\* and b\* values than those of conventional-assisted transesterified samples. Although there were no significant differences among different initial FFA concentrations in terms of L\* and a\* values, microwave-assisted transesterified OO with 10.0% FFA had significantly higher b\* values than those of OO with 2.5 and 5.0% FFA. There was a positive correlation between the L\* value and final yield ( $R^2 = 0.948$ ; L\* value =  $(0.152 \times \text{Final yield}) + 46.597$ ). Also, there was a negative correlation between the a\* value and final yield ( $R^2 = 0.803$ ; a\* value =  $(-0.044 \times \text{Final yield}) - 4.889$ ) and between the b\* value and final yield ( $R^2 = 0.985$ ; b\* value =  $(-0.4175 \times \text{Final yield}) + 77.45$ ). Therefore, although the L\* value increased by increasing final yield, both a\* and b\* values decreased. The microwave heating method showed the highest L\* value (61.50) and at the same time the lowest a\* and b\* values (9.25 and 36.50, respectively).

The physical properties of the FAMEs produced are meaningfully related to their transesterification final yield. Therefore, the physical properties of FAMEs can be used as reliable indices for predicting transesterification yield.

#### 4. CONCLUSIONS

In this study, the effects of the microwave heating method on the esterification and transesterification of HFFAOO were evaluated in comparison with the conventional heating method. By increasing the initial FFA concentration, the weight of yield, purity, and final yield of FAMEs decreased significantly. Although there were no significant differences between the

yields of MAE and CAE, the MAE reaction time was significantly lower than CAE. Also, MAT significantly increased FAME yield and simultaneously decreased reaction time in comparison with the CAT method. Therefore, the microwave heating method can be introduced as a suitable alternative method for two-step biodiesel production from different vegetable oils (olive, palm, canola, soybean, safflower, sunflower, etc.). Although the number of studies on the feasibility of novel technologies (such as reactive distillation, membrane reactor, oscillatory baffled reactor, ohmic, ultrasound, etc.) for biodiesel production is significant, detailed analyses of two-step biodiesel production from high free fatty acid waste oils using novel technologies are still needed.

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