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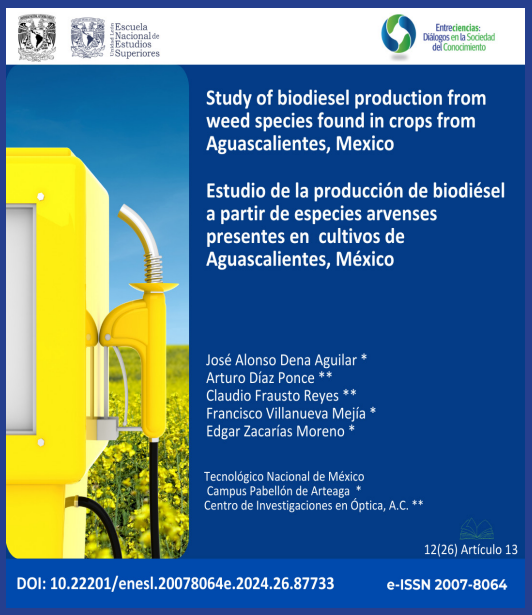

Study of biodiesel production from weed species found in crops from Aguascalientes, Mexico

Estudio de la producción de biodiésel a partir de especies arvenses presentes en cultivos de Aguascalientes, México

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Abstract

Purpose: To analyze, at a laboratory level, the biodiesel production yield by transesterification of the vegetable oil extracted from weed species growing in local crop areas in Aguascalientes, Mexico.
Methodological Design: The study evaluated the biodiesel production yield by transesterification of the vegetable oil from the weed species *Bidens ferulifolia* (Jacq.) DC., *Tithonia tubaeformis* (Jacq.) Cass., and *Bidens sambucifolia* (Cav.). Their vegetable oil is extracted through an L9(3^4) design using alcohols and alkanes as solvents. Transesterification is conducted under an L4(2^3) design, employing NaOH and methanol as a catalyst and excess alcohol, respectively. Raman Spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR) were used to characterize the biodiesel samples obtained.
Results: In the FTIR and Raman spectra of the obtained biodiesel, peaks were observed at wavenumbers 1740 cm^{-1} and 1450 cm^{-1}, respectively, corresponding to the C=O group of the conventional ester present in the fatty acid methyl esters of the biodiesel. This confirms that biodiesel was successfully produced from the studied weed species with production yields ranging between 10.3 and 15.3%.
Research limitations: This is one of the first studies to provide information on the biodiesel production capacity of the selected weed species.
Findings: Biodiesel was successfully produced through transesterifying vegetable oil extracted from the selected weed species. Further research is warranted to enhance biodiesel yields.

Keywords: Biodiesel, weed species, Bidens ferulifolia (Jacq.) DC., Tithonia tubaeformis (Jacq.) Cass., Bidens sambucifolia (Cav.).

Resumen

Objetivo: analizar a nivel laboratorio el rendimiento de producción de biodiésel por transesterificación del aceite vegetal extraído de especies arvenses crecientes en terrenos de cultivos locales en Aguascalientes, México.
Diseño Metodológico: el estudio se centró en evaluar el rendimiento de producción de biodiésel por transesterificación del aceite vegetal extraído de las especies arvenses *Bidens ferulifolia* (Jacq.) DC., *Tithonia tubaeformis* (Jacq.) Cass. y *Bidens sambucifolia* (Cav.). La extracción del aceite se realizó mediante un diseño L9(3^4) empleando alcoholes y alcanos como solventes. La transesterificación se efectuó bajo un diseño L4(2^3) empleando NaOH y metanol como catalizador y alcohol en exceso, respectivamente. Las muestras del biodiésel obtenido fueron caracterizadas por espectroscopia Raman y espectroscopia infrarroja por transformada de Fourier (FTIR).
Resultados: en los espectros de FTIR y de Raman del biodiésel obtenido se observaron picos a un numero de onda de 1740 cm^{-1} y de 1450 cm^{-1}, respectivamente, los cuales corresponden al grupo C=O del éster convencional presente en los ésteres metílicos de ácidos grasos del biodiésel. Lo anterior, confirma que se logró producir biodiésel de las especies arvenses de estudio con rendimientos de producción entre 10.3 y 15.3%.
Limitaciones de la investigación: este es uno de los primeros estudios que aporta información sobre la capacidad de producción de biodiésel de las especies arvenses seleccionadas.
Hallazgos: se logró producir biodiésel a partir de la transesterificación del aceite vegetal extraído de las especies arvenses seleccionadas. No obstante, se justifica la realización de futuras investigaciones para mejorar los rendimientos de producción de biodiésel.

Palabras clave: Biodiésel, especies arvenses, Bidens ferulifolia (Jacq.) DC., Tithonia tubaeformis (Jacq.) Cass., Bidens sambucifolia (Cav.).

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Introduction

Biofuels, primarily first or second-generation, such as biogas, bioethanol, biobutanol, and biodiesel, are generated from various organic materials that are regenerative, thus considered renewable, eco-friendly, non-toxic, and biodegradable (Cavelius *et al.*, 2023). Generally, biofuels can be produced under processes designed to enhance the processing of the biomass used, for example, through pyrolysis, anaerobic digestion, or transesterification, among other methods (Campos-Martín *et al.*, 2020). In this context, Peng *et al.* (2020) highlight the capability of using microalgae to produce biogas through anaerobic digestion, bioethanol through fermentation, and biodiesel through transesterification. Melendez *et al.* (2021) report ethanol production from sugar fermentation from sugarcane or corn. Suzihaque *et al.* (2022) examine the production of biodiesel through transesterification from used cooking oil. Specifically, biodiesel is a renewable, biodegradable biofuel that emits fewer pollutants and is thus considered an alternative energy source to replace petroleum-based fuels partially (Graziottin *et al.*, 2021). Chemically, biodiesel can be produced by transesterification of triglycerides from vegetable oils or animal fats via a basic or acid catalysis reaction in the presence of short-chain alcohols like methanol or ethanol (Fazil-Khan *et al.*, 2020; Woo *et al.*, 2021; Unruean, Nomura and Kitiyanan, 2022). Specifically, biodiesel can be manufactured from triglycerides of various biomasses such as palm, canola, corn, jatropha, sunflower, and jojoba oils (Adekunle *et al.*, 2020; Woo *et al.*, 2021; Unruean, Nomura, and Kitiyanan, 2022). Commonly, homogeneous catalysts like NaOH, KOH, or CH_3NaO are used to conduct transesterification catalysis because they offer the advantage of high catalytic activity but also present the disadvantage of potentially forming soaps through saponification reactions (Unruean, Nomura, and Kitiyanan, 2022). Additionally, reaction conditions (type of catalyst, reaction temperature, and type of excess alcohol) are crucial for achieving better biodiesel production yields. For instance, Chamola *et al.* (2019) achieved conversions of microalgae oil into biodiesel up to 87.4% using NaOH, 50 °C, and methanol. Onukwuli *et al.* (2020) obtained conversion yields of *Azadiracchta indica* seed oil into biodiesel between 80 and 90% using NaOH, between 55 and 65 °C, and methanol. Hoseini *et al.* (2021) reported conversion yields of *Chrozophora tinctoria* seed oil into biodiesel of 84% using KOH, 45 °C, and methanol. Jain *et al.* (2023) reported conversion yields of 92% from predominantly used cooking oil into biodiesel using NaOH, 50°C, and methanol. Ramírez *et al.* (2023) studied the conversion of recycled vegetable oil into biodiesel using different concentrations of NaOH and KOH, 55 °C, and methanol, achieving yields between 78.18 and 93.31%. In this context, vegetable oil derived from weed species could be an option for biodiesel production. Weed plants, also known as weeds, are wild plants that grow in agricultural fields and are considered harmful species. Without proper control, they can reduce the yield and quality of crops or act as hosts for pests and diseases (Negrín *et al.*, 2007; Blanco and Leyva, 2010; Vargas-Batis *et al.*, 2014). Despite being temporary species, their abundant presence has been reported in corn or wheat cultivation areas (Sánchez-Blanco and Guevara-Féfer, 2013; Ahmad *et al.*, 2016; Guzmán-Mendoza *et al.*, 2022). For this reason, certain species are attributed with a productive potential for biodiesel because they naturally exhibit high densities per unit area, which allows for significant seed yield values that facilitate the extraction of their oil and subsequent conversion into biodiesel (Flores-Villamil *et al.*, 2018). To cite an example, the *Argemone mexicana* species shows a potential seed yield of over 200 kg/ha and an oil yield of 1315 kg/ha. (Flores-Villamil *et al.*, 2018) and where Ashine *et al.* (2023) studied the production of biodiesel from the oil of this species and achieved a conversion percentage of 99.07%. However, in the state of the art, to date, there have been no reported studies proposing the use of *Bidens ferulifolia* (Jacq.) DC., *Tithonia tubaeformis* (Jacq.) Cass. and *Bidens sambucifolia* (Cav.) as productive potential biomasses for biodiesel. This study aimed to analyze, at a laboratory level, the biodiesel production yield derived from vegetable oil extracted from weed species with high densities per unit area and annual growth in the State of Aguascalientes, Mexico. Specifically, the production of biodiesel from the vegetable oil extracted from the seeds of the weeds *Bidens ferulifolia* (Jacq.) DC. (aceitilla amarrilla), *Tithonia*

tubaeformis (Jacq.) Cass. (acahual), and, *Bidens sambucifolia* (Cav.) (aceitilla naranja), was studied. NaOH and methanol were used as the catalyst and excess alcohol, respectively. The characterization of the obtained biodiesel samples was conducted using Raman spectroscopy and Fourier-transform infrared spectroscopy (FTIR). The article is organized as follows: the first section describes the techniques used for weed collection, vegetable oil extraction, biodiesel transesterification, and characterization. The second section details the achieved results and the analysis of the applied characterization. Finally, the last section presents the conclusions.

Methodology

Weed Collection

In Aguascalientes, Mexico, the weed species under study exhibit germination cycles shortly after the annual rainy season, displaying high densities per unit area, which is why they were selected to validate their potential for biodiesel production. Furthermore, Hernández-Salazar (2021) estimated that for every 6 kg of *B. ferulifolia*, there is a potential seed yield of up to 0.8 kg, providing sufficient biomass for this analysis. The studied weeds were collected *in situ* during September and October in the years 2020, 2021, and 2022. The collection took place on the edges of agricultural fields adjacent to the institution hosting the project (latitude 22° 09' 40.4" N, longitude 102° 16' 27.9" W), and along the roadside of State Highway 90 at kilometer 0+400 (latitude 22° 09' 23.3" N, longitude 102° 16' 33.3" W). Both collection areas are situated in the Municipality of Pabellón de Arteaga, Aguascalientes, Mexico (latitude 22° 08' 51.4" N, longitude 102° 16' 45.6" W). In Figure 1, a collection site adjacent to a corn cultivation field, located beside the project headquarters, is depicted. The weeds share the same growth space in this illustration. The appearance of the freshly harvested studied weeds is depicted in Figure 2. The *B. ferulifolia* and *B. sambucifolia* flowers exhibit a similar physiognomy, differing from each other by the number and color of the petals. The *T. tubaeformis* flower is nearly twice the size of the *B. ferulifolia* and *B. sambucifolia* and resembles sunflowers in its physiognomy. During the harvest of the weeds, the plants were collected from the root, but only the flowers were separated and subjected to natural drying in the shade. They were then stored until needed.

Figure 1. Collection site adjacent to agricultural cultivation fields

Source: Author's own elaboration.

Figure 2. Studied weed species. (a) *Bidens ferulifolia* (Jacq.) DC. (aceitilla amarrilla), (b) *Tithonia tubaeformis* (Jacq.) Cass. (acahual), and (c) *Bidens sambucifolia* (Cav.) (aceitilla naranja)

(a) (b) (c)

Source: Author's own elaboration.

Vegetable Oil Extraction

The vegetable oil from the study weeds was extracted using the Soxhlet extraction technique, as it is the most commonly used method for extracting essential oils from seeds (Sekhar *et al.*, 2021; Omeje *et al.*, 2022). A 250 mL

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Soxhlet apparatus (condenser, extraction chamber, and round-bottom flask) and a Lab Companion HP3100 heating and magnetic stirring plate were utilized in this study. The solvents used were ACS-grade hexane PM 86.18, ACS-grade butyl alcohol PM 74.12, and 96° potable alcohol (ethyl alcohol). All reagents were procured from Merck Chemical Co. and used as received. For experimental tests, the receptacle and androecium of the flower were manually crushed to break down their spherical form and reduce the volume that the weed seed would occupy in the porous material cartridge (a thimble made of medium-pore filter paper) of the Soxhlet apparatus, but primarily used to facilitate the penetration of the solvent into the biomass matrix, allowing it to reach and extract the target oil substance effectively. The experimental tests were conducted under an orthogonal Taguchi design $L9(3^4)$ as reported by Gutiérrez-Pulido and De la Vara-Salazar (2008) and shown in Table 1. The following variables (factors) were established for study: (1) type of weed species, (2) type of solvent (alcohol or alkane), (3) amount of biomass (weed seed), and (4) year of weed harvest. Specifically, three levels were established for each factor according to the selected arrangement. Additionally, other quasi-constant operating variables were established: (a) amount of solvent - 250 mL of solvent was used in all tests; (b) operating temperature - for each test and depending on the type of solvent, the temperature was set to the boiling point of the solvent itself, i.e., 69, 119, and 78.29 °C for hexane, butanol, and ethanol, respectively; (c) particle size - that of the crushed seed (not measured), and (d) operating time - for each test and depending on the type of solvent, the extraction time of vegetable oil from the studied weeds was determined based on a specific number of siphons until no more vegetable oil was extracted, and the siphons contained clear liquids (at least 3 consecutive siphons). Upon concluding the Soxhlet extraction time, a concentrated extract, referred to as residue 1, was observed in the round-bottom flask. Residue 1 had a green or light-yellow hue (depending on the weed type) and consisted of a mixture of vegetable oil and solvent. Figure 3 illustrates the resulting appearance of residue 1 from run C1.5. In all tests with hexane, and depending on the weed species, the Soxhlet extraction times ranged between 40 and 60 minutes. For tests with ethanol and butanol, the times varied between 160 and 220 minutes, depending on the weed used. Overall, all experimental Soxhlet extraction runs produced between 155 and 205 mL of residue 1, depending on the type of weed and solvent employed. Subsequently, the residue from each experimental run was subjected to a simple distillation process. This technique has also been used to extract essential oils (Tefera *et al.*, 2018; Swathanthra and Naik, 2022) to purify (separate the solvent) and recover the oil. A Liebig (straight) condenser apparatus of 300 mm, a 250 mL round-bottom flask, and a Lab Companion HP3100 heating and magnetic stirring plate were used. The distillation operation aimed to remove the excess solvent present in residue 1, obtaining only the vegetable oil from the studied weeds. Distillation took place for average times between 30 and 45 minutes until reaching a second extract, referred to as residue 2, which contains a higher concentration of vegetable oil and a darker green or yellow color. Distillation temperatures were by the boiling point of the employed solvent. The appearance of residue 2 from run C1.5 is shown in Figure 4. On average, all experimental runs –in their distillation stage– produced between 10.5 and 19.5 mL of residue 2 (depending on the type of weed and solvent used). Residue 2 underwent transesterification tests. For this purpose, each run was repeated until a sufficient volume of residue 2 for the conversion reactions was accumulated.

Table 1. Factors and experimental levels used in the $L9(3^4)$ study design

Run	Specie	Solvent	Seed Quantity (g)	Harvest year
C1.1	*B. ferulifolia*	Ethanol	10.0	2020
C1.2	*B. ferulifolia*	Hexane	12.5	2021
C1.3	*B. ferulifolia*	Butanol	15.0	2022
C1.4	*T. tubaeformis*	Ethanol	12.5	2022
C1.5	*T. tubaeformis*	Hexane	15.0	2020
C1.6	*T. tubaeformis*	Butanol	10.0	2021
C1.7	*B. sambucifolia*	Ethanol	15.0	2021
C1.8	*B. sambucifolia*	Hexane	10.0	2022
C1.9	*B. sambucifolia*	Butanol	12.5	2020

Source: Author´s own elaboration

Figure 3. The appearance of residue 1 resulting from the Soxhlet extraction of run C1.5

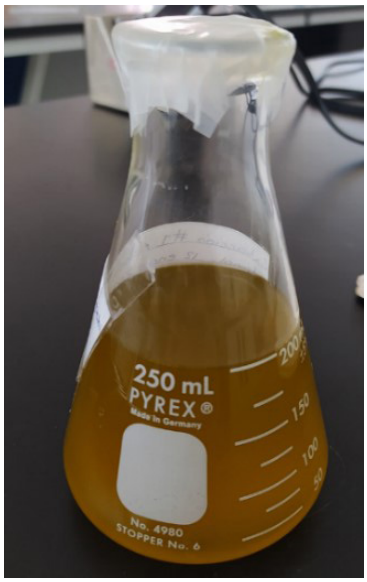

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Figure 4. The appearance of the concentrated residue 2 resulting from the distillation of residue 1 from run C1.5 (accumulated from multiple distillations)

Source: Author's own elaboration.

Yields of Obtained Biodiesel

The biodiesel yield (%) was calculated using Equation (1) as reported by Kiliç *et al.* (2013), Bateni *et al.* (2014), Bateni and Karimi (2016), and Rodríguez-Bustamante *et al.* (2022).

$$Biodiesel\ yield\ (\%) = \frac{Produced\ biodiesel\ (mL)}{Raw\ oil\ (mL)} \times 100$$

Transesterification of Weed Specie

The transesterification process was realized through chemical reactions to obtain alkyl esters of fatty acids derived from the vegetable oil obtained in the extraction and distillation tests (residue 2). The transesterification methodology was defined based on the work of Chamola *et al.* (2019), Onukwuli *et al.* (2020), Hoseini *et al.* (2021), Jain *et al.* (2023), and Ramírez *et al.* (2023). NaOH solution 50% in H_2O (industrial grade, PM 40.0, ρ=1.52 g/mL) was used as a catalyst, and ACS grade methanol (PM 32.04) was used as the excess alcohol. The reagents were procured from Golden Bell and used as received. Transesterification reactions were conducted in triplicate under an orthogonal Taguchi design $L4(2^3)$ as reported by Gutiérrez-Pulido and De la Vara-Salazar (2008), with the following variables (factors): (1) volume of vegetable oil (mL), (2) volume of excess alcohol (mL), and (3) volume of the catalyst (µL). Two levels were set for each factor. Table 2 specifies the factors and experimental levels defined for the transesterification reactions. To carry out the reactions, a 100 mL glass reactor (precipitation beaker) equipped with a Brannan Lo-Tox Blue immersion thermometer and a Lab Companion HP3100 heating and magnetic stirring plate was used. To determine the quantities of excess alcohol and catalyst, the recipe for producing biodiesel from virgin vegetable oil defined by Varty and Lishawa (n.d.) was employed. The following methodology was used in all transesterification experimental tests. Firstly, the volume of vegetable oil was heated to a temperature of 55 ±1 °C, and once the working temperature was reached, magnetic stirring was applied (at a turbulent regime of 100 rpm to ensure homogeneous mixing), and immediately excess alcohol followed by the catalyst was added. The complete reaction was maintained for 30 minutes. After the operation

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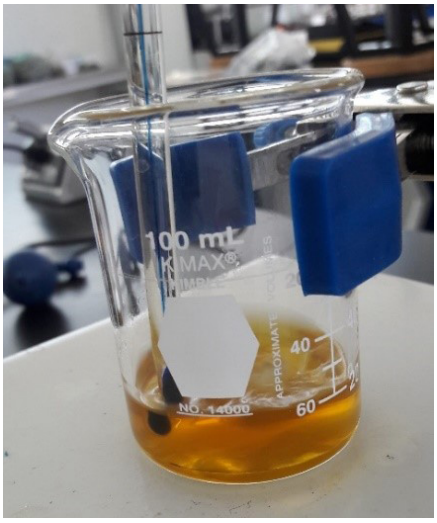
time, the final liquid residue was allowed to settle for 48 hours in a separation funnel. At the end of the resting period, the formation of two or three layers was observed, clearly separated by density differences. These layers correspond to byproducts of the transesterification process, such as glycerin (also called glycerol) and the biodiesel itself (Vávra, Hájek, and Kocián, 2021; Menéndez *et al.*, 2023; Analuisa *et al.*, 2024). At this stage, two or three layers were identified: (a) a lower first layer that mostly corresponded to the formation of glycerin with some traces of concentrated saponified compounds, (b) an intermediate second layer that is a mixture of glycerin and other obtained saponification compounds, and (c) an upper third layer (the one of interest) where the achieved biodiesel was deposited. After the separation time, only the upper layer of interest (liquid with a pH between 10.0 and 11.0) was collected, and it underwent a washing test with distilled water to remove the excess unreacted catalyst, saponified residues still present, and reach a neutral pH. Finally, any trace of water was separated from the resulting final liquid. The final product was characterized. The appearance of the transesterification reaction from run C2.2 using ragweed vegetable oil is shown in Figure 5.

Table 2. Factors and experimental levels used in transesterification

Run	Vegetable oil volume (mL)	Excess alcohol volume (mL)	Catalyst volume (µL)
C2.1	20.0	4.0	46.0
C2.2	20.0	6.0	69.0
C2.3	30.0	4.0	69.0
C2.4	30.0	6.0	46.0

Source: Author´s own elaboration

Figure 5. Transesterification reaction from run C2.2 with *T. tubaeformis* vegetable oil obtained from run C1.5



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Characterization of biodiesel samples

The obtained biodiesel samples were characterized using Fourier-Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy techniques. Infrared spectra of all biodiesel samples were obtained using an Agilent Cary 670 FTIR spectrophotometer. The infrared spectra were recorded in the range of 3500-500 cm^{-1} with a resolution of 0.1 cm^{-1} and 20 scans. Isobutanol was used to clean and purify the crystal of the equipment between each measurement, and the results of each scan were processed with the equipment's proprietary computer software. Raman spectra were obtained using a Micro Raman system with an excitation wavelength of 632.8 nm and recorded in the range of 3500-500 cm^{-1} with a resolution of 1 cm^{-1}. In both instruments, the samples were analyzed without any prior treatment.

Results and discussion

Weed-to-Biodiesel Tests

For this study, the number of layers formed was established as the first indication of biodiesel production. Specifically, weeds treated with hexane and butanol

solvents displayed three separation layers, while those treated with ethanol only showed two layers. These results can be explained in terms of the polar or non-polar nature of the solvents used during the extraction stage. Predominantly, vegetable oils are considered non-polar, even though they may contain slight traces of polar compounds (Ramírez-Botero *et al.*, 2012) On the other hand, hexane and ethanol are non-polar and polar solvents, respectively (Calle-Chumo *et al.*, 2023). In the case of butanol, it is mainly considered non-polar due to its predominant long C_4H_9 chain although it can also behave slightly as a polar compound due to the OH group in its molecule (ChemicalBook, 2024). This is why hexane and butanol were able to extract the triglycerides from the vegetable oil in the seeds of the studied weed species with greater dissolution affinity, ultimately resulting in the formation of sufficient biodiesel esters relative to the three layers of interest. Figure 6 shows two samples with three layers. Only experiments resulting in three separation layers underwent washing tests. The washing process is one of the main wet purification methods for biodiesel, which effectively removes excess catalysts and other impurities present in the samples. This operation is characterized by requiring several washing cycles to achieve neutrality. It is easy to implement because, throughout the process, the wash water settles at the bottom while the biodiesel remains on the surface (Polishchuk *et al.*, 2020). Consequently, in this study, the physical property of immiscibility of the sample during the washing tests was established as the second indicator of biodiesel production. Only samples initially treated with hexane (from the extraction stage) showed immiscibility with the washing water. All experiments conducted with butanol were miscible with the washing water. In this latter case, even though three layers of separation were previously observed, the polar part of the butanol molecule played a decisive role in causing the biodiesel samples associated with this alcohol to completely dissolve in the wash water (as a polar compound). This highlights the complex interactions between the chemical nature of the solvents used in biodiesel production and their behavior during purification processes. An example of an experimental sample resulting in immiscibility is shown in Figure 7, where the separation of liquids is observed. All immiscible samples underwent spectroscopic analysis, so only weeds subjected to extraction with hexane were characterized.

Figure 6. Test resulting in three layers and treated with hexane. (a) *T. tubaeformis* biodiesel obtained from runs C1.5 and C2.2, and (b) *B. ferulifolia* biodiesel obtained from runs C1.2 and C2.2

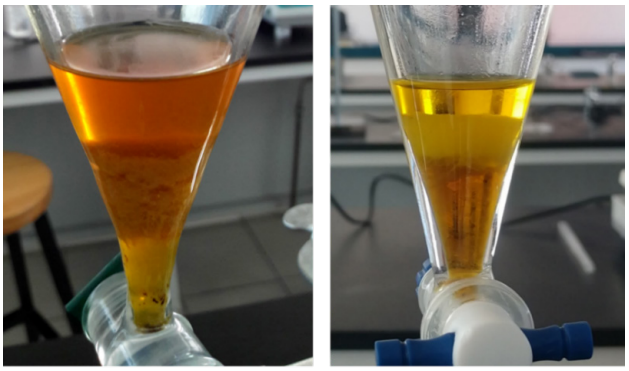

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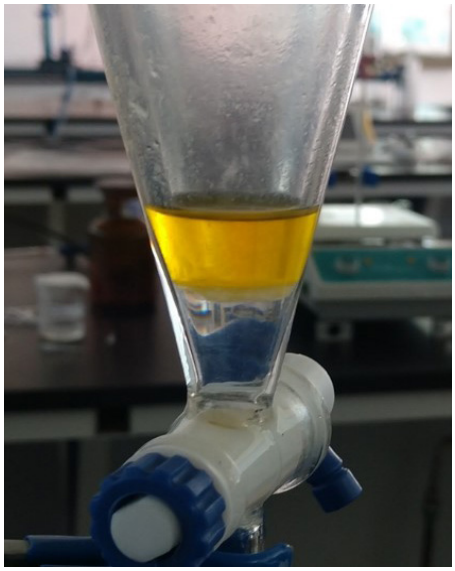
Figure 7. Immiscible sample of *B. ferulifolia* biodiesel obtained from runs C1.2 and C2.2



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Characterization of Weed Biodiesel Samples

Every one FTIR and Raman spectra of the final biodiesel samples were analyzed. The FTIR spectra of *B. ferulifolia* (aceitilla amarrilla), *T. tubaeformis* (acahual), and, *B.*

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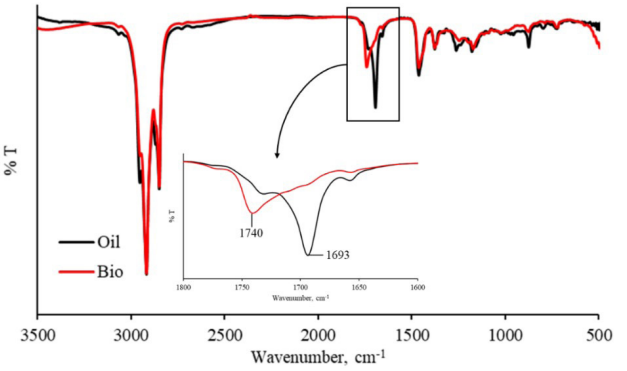
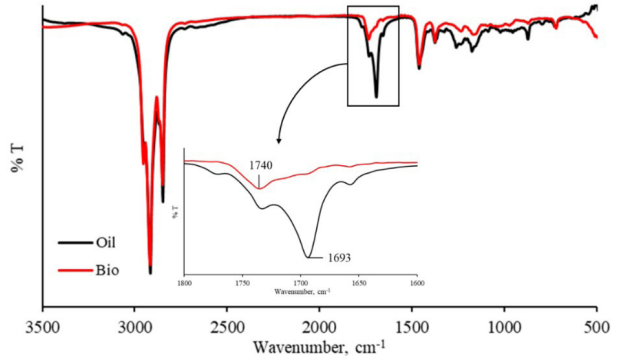
sambucifolia (aceitilla naranja), both from their vegetable oil and the biodiesel obtained, are shown in Figures 8, 9, and 10, respectively. Analyzing Figure 8, the characteristic biodiesel peak at 1740 cm^{-1} is observed, corresponding to the stretching of the C=O in the methyl esters of fatty acids present in biodiesel (Lafont *et al.*, 2011; Tariq *et al.*, 2011; O'Donnell *et al.*, 2013; Cunha *et al.*, 2017; Atabani *et al.*, 2019; Kamaronzaman *et al.*, 2020). Additionally, a peak at 1693 cm^{-1} in the vegetable oil spectrum corresponds to the stretching of the carbonyl group C=O (Gore, 1972; Baeten *et al.*, 2005; Silverstein *et al.*, 2005; Concha-Herrera *et al.*, 2009) present to the carbonyl group in the triglycerides of the vegetable oil samples. These two peaks were the most characteristic identified in each of the spectra. Specifically, the absence of the peak at 1693 cm^{-1} in the biodiesel spectra confirmed the conversion of the vegetable oil from the studied weed species into the desired biofuel. In the case of Figure 9, the same signals at 1740 and 1693 cm^{-1} were observed. For Figure 10, the biodiesel peak at 1740 cm^{-1} is clearly shown, but there is also a peak at approximately ~1702 cm^{-1}, which, due to its location, corresponds to the C=O of the triglycerides in the vegetable oil. In general, the analysis and comparison of the FTIR spectra of the biodiesel and the vegetable oil –from each of the study weeds– confirmed that biodiesel was successfully obtained using the proposed methodology. In Table 3, other bands and peaks present in the FTIR spectra of the study samples are described. On the other hand, Figure 11 displays the Raman spectra of vegetable oil and biodiesel from *B. ferulifolia*. The vibrations of the functional groups present in the Raman spectra are specified in Table 4. Analyze Figure 11, Raman vibrations in 1000 to 1750 cm^{-1} region are observed in the biodiesel spectrum but absent in the vegetable oil spectrum, indicating the conversion of vegetable oil into biodiesel. The main signal is the vibration at 1450 cm^{-1} corresponding to the stretching of the methyl ester C=O in biodiesel (Socrates, 2001; Firdous *et al.*, 2016). The Raman spectra of *T. tubaeformis* and *B. sambucifolia* are shown in Figure 12. The Raman spectra of these two weeds (in comparison with the spectrum of *B. ferulifolia*) were affected by a very high fluorescence background, which prevented the visibility of the Raman peaks in the samples. In the case of *T. tubaeformis*, there are no visible Raman peaks, while in *B. sambucifolia*, they are almost imperceptible. This is because the photons from the excitation laser in the Raman system have enough energy to generate the phenomenon of fluorescence more efficiently.

Figure 8. FTIR of vegetable oil (Oil) [obtained from run C1.2] and biodiesel (Bio) [obtained from run C2.2] from *B. ferulifolia*



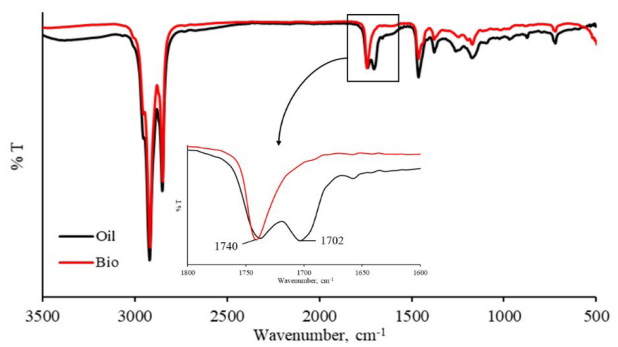
Source: Author's own elaboration.

Figure 9. FTIR of vegetable oil (Oil) [obtained from run C1.5] and biodiesel (Bio) [obtained from run C2.2] from *T. tubaeformis*



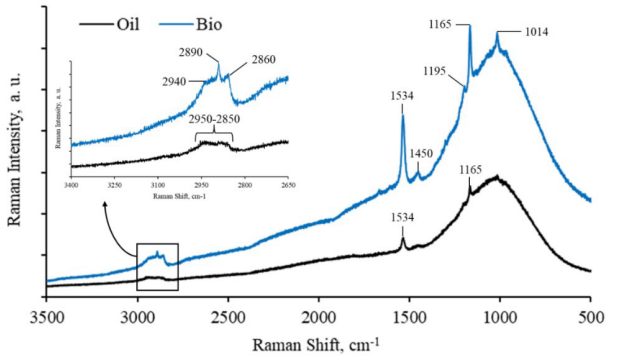
Source: Author's own elaboration.

Figure 10. FTIR of vegetable oil (Oil) [obtained from run C1.8] and biodiesel (Bio) [obtained from run C2.2] from *B. sambucifolia*



Source: Author's own elaboration.

Figure 11. Raman spectra of vegetable oil (Oil) [obtained from run C1.2] and biodiesel (Bio) [obtained from run C2.2] from *B. ferulifolia*



Source: Author's own elaboration.

Table 3. FTIR frequencies of common functional groups present in biodiesel samples obtained from *B. ferulifolia*, *T. tubaeformis*, and *B. sambucifolia*

Frequency (cm^{-1})	Type of functional group vibration
2848-2955	Stretching of CH_3 and CH_2 aliphatic groups
1461	Bending in the CH_2 plane
1377	Asymmetric stretching of CH_2
1235-1261	Asymmetric stretching of C–O–C
1170	Stretching and bending of C–CO–C
873	Stretching of C=O
728	Stretching of CH_2
720	Bending in the CH_2 plane

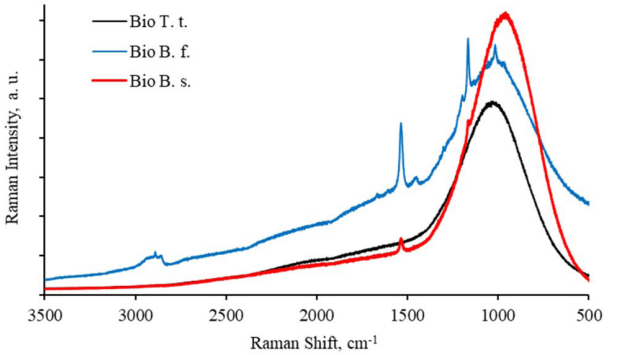
Source: Author's own elaboration

Table 4. Raman vibrations of present functional groups present in *B. ferulifolia* biodiesel spectra

Region (cm^{-1})	Type of functional group vibration	Present in the spectrum of
2850-2950	Combination of C–H stretches	Vegetable oil
2940	Asymmetric stretching CH_2	Biodiesel
2890	Stretching CH	Biodiesel
2860	Symmetric stretching CH_2	Biodiesel
1535	Stretching C=C	Oil and Bio
1450	Methyl ester C=O attached to asym. CH_3 def. vibration	Biodiesel
1195	$–C(CH_3)_3$ skeletal vibration	Biodiesel
1165	Stretching C–C	Oil and Bio
1014	Rocking $–CH_3$	Biodiesel

Source: Author´s own elaboration

Figure 12. Comparison of Raman spectra of biodiesel from *B. ferulifolia* (Bio B. f.), *T. tubaeformis* (Bio T. t.), and *B. sambucifolia* (Bio B. s.)



Source: Author's own elaboration.

DOI:10.22201/enesl.20078064e.2024.26.87733

Biodiesel Yield Percentages

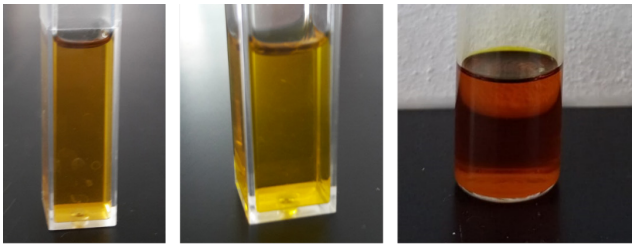
In Table 5, the average biodiesel yield percentages obtained under the proposed study methodology are described. For any weed (regardless of the year of its harvest) treated with hexane –from the extraction stage– biodiesel was efficiently obtained with average yields ranging from 10.3 to 15.3%. No other study was found in the literature has analyzed the biodiesel production potential of the selected weed species as done in this work. However, other studies using NaOH as a catalyst reported biodiesel production yields above 80% (Chamola *et al.*, 2019; Onukwuli *et al.*, 2020; Jain *et al.*, 2023; Ramírez *et al.*, 2023). Therefore, while the objective of producing biodiesel was achieved in this study, further analysis of operating conditions is necessary to optimize the process. For example, using a heterogeneous catalyst, employing purer solvents, establishing different experimental designs, and performing a pretreatment on the seed to facilitate greater solvent penetration into the seed's cellular matrix could be considered. Figure 13 illustrates the final appearance of the biodiesel obtained from each weed. In the case of biodiesel from *B. ferulifolia* and *T. tubaeformis*, their yellow colors were very similar, ranging from honey-yellow to canary-yellow. Conversely, the color of the biodiesel from *B. sambucifolia* resulted in a brownish hue.

Table 5. Average Biodiesel Yields Produced

Weed	Seed quantity (g)	Amount of produced biodiesel (mL)	Biodiesel yield (%)
B. sambucifolia	10.0	2.6	10.3
B. ferulifolia	12.5	3.4	13.5
T. tubaeformis	15.0	3.7	15.3

Source: Author´s own elaboration

Figure 13. The appearance of biodiesel obtained from weeds (a) *B. ferulifolia*, (b) *T. tubaeformis*, and (c) *B. sambucifolia*



Source: Author's own elaboration.

Conclusions

Through the proposed methodology, biodiesel was successfully obtained from the vegetable oil of the weed species *Bidens ferulifolia* (Jacq.) DC. (aceitilla amarrilla), *Tithonia tubaeformis* (Jacq.) Cass. (acahual) and *Bidens sambucifolia* (Cav.) (aceitilla naranja). This was achieved using hexane as the solvent, NaOH as the catalyst, methanol as the excess alcohol, a transesterification temperature of 55 ±1°C, stirring at 100 rpm, an $L9(3^4)$ design for the extraction, and an $L4(2^3)$ design for the transesterification. In the case of samples treated with hexane from the extraction, three separation layers were observed, immiscibility with the wash water, and FTIR and Raman spectroscopy analysis confirmed the production of characteristic biodiesel esters. On the other hand, biodiesel was not obtained using ethanol and butanol solvents under the established operating conditions. On average, biodiesel yield percentages ranged between 10.3 and 15.3%. FTIR characterization confirmed the production of biodiesel, as a peak at 1740 cm^{-1} corresponding to the carbonyl group (C=O) of the methyl esters present in biodiesel was observed. Raman characterization also confirmed the production of the biofuel due to the presence of vibration at 1450 cm^{-1} corresponding to the methyl ester C=O stretch of biodiesel. However, the proposed methodology needs to be optimized to achieve better yields through the use of a different catalyst, employing purer solvents, defining other experimental designs, and providing pre-treatment to the seed before oil extraction (future work).

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