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# Chemical composition of biodiesel produced *in situ* with *Salvinia molesta* DS Mitchell (Salviniaceae) by ethylic and methylic route

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**ABSTRACT.** Macrophytes are aquatic plants that can cause environmental and economic damage due to their rapid growth in eutrophicated environments; however, this characteristic makes these biomasses promising alternatives for biodiesel production. Thus, this study aims to characterize and evaluate the chemical composition of the biodiesel produced from the macrophyte *Salvinia molesta* DC Mitchell (Salviniaceae). The biodiesel production was carried out *in situ* through the ethyl and methyl process. Attenuated total reflectance-Fourier transform infrared spectroscopy (FTIR-ATR) and gas chromatography with a flame ionization detector (GC-FID) were used to characterize the product. A commercial sample was also analyzed for comparison purposes. The biofuel produced with ethanol and methanol showed characteristic peaks between 900 to 1300 $cm^{-1}$ and 1750 to 1735 $cm^{-1}$ in the FTIR-ATR. Both samples showed less unsaturation degree compared to the commercial sample, with 34.44% of monounsaturated compounds (MUFA) and 36.73% of polyunsaturated compounds (PUFA) for methylic biodiesel, 34.79% of MUFA and 36.89% of PUFA for ethylic biodiesel, and 55.34% of MUFA and 24.14% of PUFA for commercial biodiesel. Samples produced by both routes showed similar chemical composition, with higher contents of saturated compounds than the commercial sample. The average chain size and the number of double bonds are smaller for *S. molesta* samples, 17.38 and 1.15 for *S. molesta* biodiesels and 17.65 and 1.41 for commercial biodiesel, respectively. The chemical composition of *S. molesta* biodiesel demonstrates the potential to be an alternative to commercial biodiesel.

**Keywords:** direct transesterification; fatty acid esters; gas chromatography; Infrared spectroscopy.

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## Introduction

The idea of vegetable oils as fuel is old, being tested by Rudolf Diesel (inventor of the diesel engine), as pointed out by Singh et al. (2020). However, it is impractical because of its physicochemical characteristics and the generation of toxic components from glycerol (Öztürk, Can, Usta, & Yücesu, 2020). Nonetheless, it is possible to employ vegetable oils by converting them into biodiesel through the transesterification reaction between alcohols and triglycerides (Lôbo, Ferreira, & Cruz, 2009).

Biodiesel has advantages over diesel because it is a biodegradable, renewable, low-polluting source, contains low sulfur content, and is free of aromatic compounds (Kozlowski et al., 2019). Biodiesel has lower energy per mass compared to diesel due to the presence of oxygen. Still, its density makes the energy content 5 to 6% lower than conventional diesel (Hoekman, Broch, Robbins, Ceniceros, & Natarajan, 2012). A method that reduces production costs is *in situ* transesterification, which avoids the lipid extraction step, with methods involving fewer steps (Park, Park, Lee, & Yang, 2015).

The growing demand for biodiesel production has resulted in the search for alternative biomasses that can be used for the production of renewable fuel (Oliveira Junior, Konradt-Moraes, Castro, & Mascarenhas-Santos, 2021; Leite et al., 2024). Studies exploring the potential of macrophytes (Oliveira Junior et al., 2021), a group of aquatic plants with a rapid growth rate (Miranda et al., 2016), have not been conducted with this

aim. Such plants can be cultivated in wastewater as they possess phytoremediation capacity (Rodrigues & Orlandelli, 2018). Cultivation in eutrophicated water significantly increases biomass, enabling its use in producing fertilizer, biofuel, and fish feed (Mubarak, Shaija, & Suchithra, 2021).

*Salvinia molesta* DS Mitchell (Salviniaceae) is a specie of macrophytes. Native to South America, it has become endemic in tropical countries (Moozhiyil & Pallauf, 1986). It shows optimal growth at 30ºC but can grow between 10 and 40ºC. However, its introduction into different biomes as an ornamental plant has led to infestations in several rivers and lakes worldwide (Lal, 2016).

Its fast proliferation makes it the aquatic plant with the most significant social, environmental, and economic impact; it can hold disease vectors (Naheed et al., 2021) and adapt to different local climates (Julien, Hill, & Tipping, 2009). Nevertheless, its use in treating contaminated water (phytoremediation) has been widely studied (Garlich et al., 2021; Musafa & Hayder, 2021).

Recently, Mubarak, Shaija, and Suchithra (2016) optimized the extraction of lipids from *S. molesta*, and Mubarak et al. (2021) analyzed the effect of *S. molesta* biodiesel blended with diesel on performance and emission after combustion. Yet, there are still no reports in the literature on the *in situ* production of biodiesel from this macrophyte.

Methanol is the most used alcohol for biodiesel production due to its lower price, easy industrial recovery of excess reactants, and better conversion in conventional reactions (Safar & Thomas, 2007; Rodrigues, 2021). Ethanol can also be used, presenting the advantage of being renewable and that Brazil has an expressive production of this product (Lima et al., 2007).

Within this context, the present study aimed to characterize the biodiesel produced from ethyl and methyl methods of *S. molesta* using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (FTIR-ATR) and gas chromatography with flame ionization detector (GC-FID) analysis.

## Material and methods

### Macrophyte sampling

*S. molesta* was collected in the Antenor Martins Municipal Park, in the city of Dourados, Mato Grosso do Sul state, Brazil (S 22°13'18' and W 54°48'23'), at 430 m altitude, and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, Brazil) under number A77F799.

The collection was performed with the help of a 15 L plastic bucket, from which the macrophytes were carefully removed and placed in another container that held lake water to be transported. The samples were washed with running water to remove organic debris from the leaves and rhizomes, dried in an oven at 60ºC until constant mass, and then ground. The biomass was stored in plastic containers at room temperature and no light.

### Production of biodiesel by methyl and ethyl route

We used the methodology described by Johnson and Wen (2009) with adaptations. For biodiesel production, each gram of sample was left in contact with a mixture of 20 mL of hexane, 3 mL of sulfuric acid, and 17 mL of methanol at 90°C and magnetic stirring for 1 hour. Finally, 5 mL of hexane and 2 mL of distilled water were added, the organic part was collected, and the solvent was removed at 60ºC.

### Infrared analysis

First, Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (FTIR-ATR) was used to verify the absorption peaks characteristic of fatty acid methyl esters. For this purpose, a spectrophotometer Thermo Nicolet Nexus 670 cooled with liquid nitrogen was employed, with detection in the region of 4000 - 400 $cm^{-1}$. In triplicate, a 30 µL aliquot was placed in an Attenuated Total Reflectance cell, performing 64 scans at a resolution of 4 $cm^{-1}$.

### Chromatographic analysis

The chemical composition was analyzed by gas chromatography (GC) in a Thermo Scientific chromatograph (Focus GC, San Jose, CA, USA), equipped with a SUPELCOWAX 10 capillary column (30 m length, 0.32 mm internal diameter and 0.25 µm thickness), using hydrogen as the carrier gas, with a constant flow of 1.0 mL per minute. The analysis was performed in triplicate.

The temperatures of the injector (split 1:20) and flame ionization detector (FID) were kept at 250°C. The oven temperature was programmed to start at 130ºC, hold for 1 minute, and then increase in the following

sequence: increase of 7ºC per minute to 170ºC, 3ºC per minute to 215ºC, and hold for 12 minutes. Finally, a 20ºC per minute rise to 215ºC was performed.

The compounds were identified by comparing them with standards purchased from Sigma-Aldrich. The peak areas determined the percentage of each compound identified.

With WMUFA being the percentage of monounsaturated compounds and WPUFA the percentage of polyunsaturated compounds, Equation 1 was used to calculate the average carbon number (LU) (Pinzi, Leiva, Arzamendi, Gandia, & Dorado, 2011; Hoekman et al., 2012).

$$LU = \sum (nC_n \times w_i)$$

Where $nC_n$ is the number of carbons in each fatty acid ester and $w_i$ is the weight percentage of each fatty acid ester. A similar method was used to calculate the average number of double bonds (MLD) by considering the number of double bonds (LD) with $w_i$, as shown in Equation 2 (Hoekman et al., 2012).

$$MLD = \sum (LD \times w_i)$$

All analyses were also performed on a commercial biodiesel (CB) sample for comparison (Standard).

### Statistical analyses

Statistical analysis was performed on the R platform (R Core Team, 2021). One-way analysis of variance (ANOVA) with Tukey posterior was performed. For yields, a t-test was done (Supplement 1).

## Results and discussion

### Yield of biodiesel

Biodiesel produced with ethanol (EB) showed a significantly higher yield (3.07 ± 0.12%) by the t-test ($p < 0.05$) compared to biodiesel produced with methanol (MB) (2.38 ± 0.10%).

Henry-Silva and Camargo (2002) obtained a lipid content of 3.8% for *S. molesta* samples from effluent treatment systems. However, Mubarak et al. (2016) reported contents between 15.36 and 19.97% when using different extraction techniques. Oliveira Junior et al. (2021) analyzed the effect of cultivating the aquatic plant *Salvinia auriculata* with biosolids and vinasse, obtaining, respectively, lipid contents of 6.82 and 7.64%, while the sample from the natural environment had only 3.28%.

In the study by Trevisan et al. (2018), aiming to exchange hexane for chloroform in the *in situ* production of biodiesel from *Chlorella vulgaris*, the former showed superior performance and a yield in ethyl esters of 13.77% in a reaction time of 150 minutes at 90°C. The maximum yield in ethyl esters reached with chloroform was 7.54% at 75°C, with 60 minutes of reaction.

Thus, it is possible to confirm that factors in cultivation (Oliveira Junior et al., 2021), complementary techniques (Mubarak et al., 2016), and temperature employed (Trevisan et al., 2018) affect the lipid contents, even when the same species are used in the studies.

The higher yield of the ethyl route has even more advantages since the ethanol comes from renewable sources (Sameeroddin, Deshmukh, Viswa, & Sattar, 2021). Nevertheless, future studies are needed to optimize the process. Leite et al. (2024) also obtained better yields for biodiesel produced with ethanol for species *Eichhornia crassipes* (Mart.) Solms by *in situ* transesterification, ranging from 5.02 to 9.74%.

### Infrared analysis

The mid-infrared spectroscopy is used to analyze biodiesel, where characteristic peaks of this biofuel are assessed, mainly those associated with carbonyl and ester bonds (Guerreiro, Pinto, Aguiar, & Ribeiro, 2008). The spectra obtained for methyl (MB) and ethyl (EB) biodiesels from *S. molesta* were similar to those obtained for commercial biodiesel (CB), also called standard biodiesel in this work (Figure 1).

In the 1800-1700 $cm^{-1}$ region, the stretching of triacylglycerols (C=O) is attributed to esters, according to Soares et al. (2008). According to Aliske, Zagonel, Costa, Viega, and Saul (2007), the carbonyl can vary in position depending on the alcohol used for transesterification. As per Figure 1, the carbonyl peaks are at 1741.4 $cm^{-1}$ for the standard and methyl biodiesel. In ethyl biodiesel, the carbonyl appeared at 1737.5 $cm^{-1}$.

The presence of peaks associated with methyl biodiesel in the commercial biodiesel sample is consistent once the Brazilian standard is produced mainly with methanol due to the lower cost (Rodrigues, 2021). The

peaks obtained from the samples of this study, on the other hand, indicate a good conversion of triglycerides to fatty acid esters for both alcohols studied.

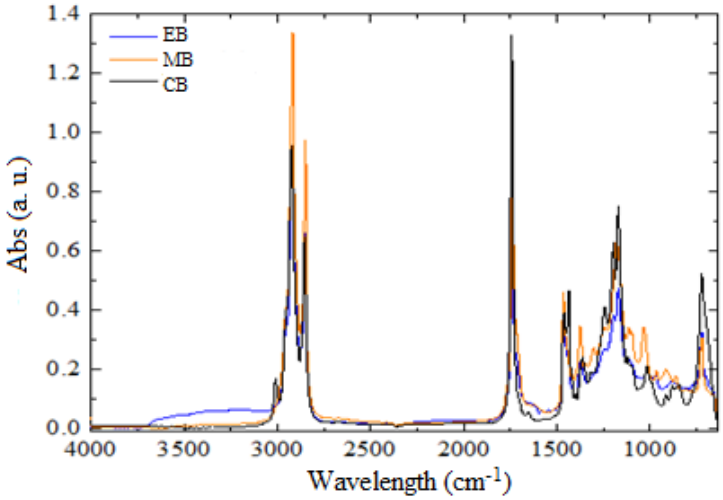


**Figure 1.** Absorption spectrum of biodiesel produced by ethyl (EB) and methyl (MB) route and commercial biodiesel (CB) (Standard). Source: The authors.

Guerreiro et al. (2008) described that the region between 1750 and 1735 $cm^{-1}$ is typical of the C-O bonds of esters and is used to identify the presence of biodiesel. The authors also reported that the absorption between 1300 and 1000 $cm^{-1}$ is associated with asymmetric vibrations coupled to the C-O group of esters. We can see that all samples presented peaks in this region (Figure 1).

According to Wongjaikham et al. (2021), the region from 900 to 1300 $cm^{-1}$, called the "fingerprint region" of the compounds, comprehends the absorption bands referring to the different vibrations of the esters that constitute biodiesel. Accordingly, Rabelo, Ferraz, Oliveira, and Franca (2015) defined the O-$CH_3$ stretching represented by the absorbance at 1196 $cm^{-1}$ as typical of biodiesel.

The biofuel produced by the methanolic route showed a peak at 2850.3 $cm^{-1}$, being associated with symmetric vibration of the C-H bond of the methyl group, indicating the formation of methyl esters, according to Elumalai and Sakthivel (2013). The presence of peaks that are characteristic of biodiesel suggests that the *in situ* transesterification reaction occurred in both chemical routes studied.

## Chromatographic analysis

The study of the chemical composition of biodiesel is essential as it relates to the physicochemical characteristics of the product (Nascimento et al., 2012). Both routes for biodiesel production from *S. molesta* culminated in the predominance of esters of oleic (C18:1), palmitic (C16:0), linoleic (C18:2), and linolenic (C18:3) fatty acids. At the same time, the commercial sample (CB) showed mostly esters of linoleic (C18:2), oleic (C18:1), and palmitic (C16:0) fatty acids (Table 1).

**Table 1.** Chemical composition obtained by gas chromatography of biodiesels.

| FAE | Correspondent fatty acid | CB (%) | EB (%) | MB (%) |
|---|---|---|---|---|
| C8:0 | Caprylic Acid | 0.12 ± 0.01 | 0.45 ± 0.01 | 0.51 ± 0.01 |
| C10:0 | Capric acid | 0.34 ± 0.01 | 0.91 ± 0.02 | 0.79 ± 0.01 |
| C12:0 | Lauric Acid | 0.18 ± 0.01 | 0.33 ± 0.01 | 0.45 ± 0.01 |
| C14:0 | Myristic acid | 1.08 ± 0.03 | 0.93 ± 0.01 | 1.03 ± 0.02 |
| C16:0 | Palmitic acid | 15.68 ± 0.13 | 27.79 ± 0.14 | 27.68 ± 0.18 |
| C16:1 | Palmitoleic acid | 0.25 ± 0.01 | 1.56 ± 0.04 | 1.49 ± 0.02 |
| C17:0 | Margaric acid | 0.26 ± 0.01 | 0.89 ± 0.01 | 0.66 ± 0.01 |
| C18:0 | Stearic Acid | 5.04 ± 0.05 | 1.69 ± 0.04 | 1.62 ± 0.03 |
| C18:1 | Oleic Acid | 20.29 ± 0.19 | 26.37 ± 0.21 | 26.89 ± 0.18 |
| C18:2 | Linoleic Acid | 45.56 ± 0.16 | 17.78 ± 0.15 | 17.56 ± 0.13 |
| C18:3 | Linolenic acid | 9.78 ± 0.07 | 17.01 ± 0.12 | 16.88 ± 0.14 |
| C20:0 | Arachidic acid | 0.48 ± 0.01 | 2.34 ± 0.05 | 1.98 ± 0.04 |
| C22:0 | Behenic acid | 0.51 ± 0.01 | 1.56 ± 0.02 | 2.01 ± 0.06 |
| C24:0 | Erucic acid | 0.45 ± 0.01 | 0.45 ± 0.01 | 0.45 ± 0.01 |

FAE = fatty acid esters; CB = commercial biodiesel; MB = methyl biodiesel; EB = ethyl biodiesel. Source: The authors.

The levels obtained show that the solvent used *in situ* for the transesterification did not affect the chemical composition of biodiesel from *S. molesta*; however, both partially diverged from the commercial sample. According to Hoekman et al. (2012), conventional oils used in transesterification typically show a predominance of C16:0, C18:0, C18:1, C18:2, and C18:3, also the compounds observed for *S. molesta* biodiesels.

Mubarak et al. (2016) obtained a different chemical composition for biodiesel produced with the oil extracted from *S. molesta*, finding higher levels of esters of palmitoleic acid (C16:1). Still, in their study, significant concentrations of C18:1 and C16:0 were also identified. Venu, Venkataraman, Purushothaman, and Vallapudi (2019) analyzed biodiesel composition from another aquatic plant, *Eichhornia crassipes*, obtaining high C16:1 content. Leite et al. (2024) also conducted a biodiesel study on *E. crassipes*, identifying C18:2, C18:1, and C16:0 as the primary compounds.

The *in situ* production may induce the degradation of the C16:1 compound; this fact could be associated with the reduced oxidative stability of unsaturated compounds (Knothe, 2007), justifying the low yield obtained in the reaction. In contrast, the oil from the macrophyte *Azolla filiculoides* does not present high contents of C16:1 (Brouwer, van der Werf, Schluepmann, Reichart, & Nierop, 2016), while Miranda, Liu, Rochfort, and Mouradov (2018) did not report this fatty acid ester in biodiesel from *A. filiculoides*. On the other hand, Gusain, and Suthar (2017) analyzed the chemical composition of *Lemna gibba*, *Lemna minor*, *Pistia stratiotes*, and *Eichhornia sp.* and described no presence of C16:1.

Within this context, further studies are needed to verify if there is indeed a loss of C16:1 in the production process or if the conditions in cultivation induce lower production of triglycerides associated with this fatty acid, given that there are reports of significant amounts of this compound in *S. molesta* (Mubarak et al., 2016).

The contents of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) biodiesel compounds produced with *S. molesta* were similar for the two routes studied (Figure 2). However, the commercial biodiesel showed lower SFA and MUFA contents, while PUFA was higher (Figure 2). This result should be associated with the abundance of C18:2 in the commercial sample (CB).

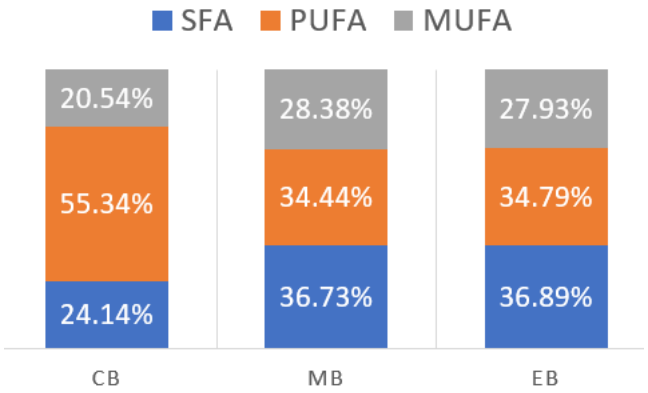


**Figure 2.** Composition in terms of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) compounds of commercial biodiesel (CB) and those produced with *S. molesta* by methyl (MB) and ethyl (EB) routes. Source: The authors.

According to Ramos, Fiorucci, Cardoso and Silva (2020), the ratio of MUFA/PUFA is associated with the oxidative stability of the sample, and Ramadan (2013) points out that higher levels of MUFA compared to PUFA induce better oxidative stability by avoiding auto-oxidation, in this sense a higher MUFA/PUFA indicates greater oxidative stability. Thus, the biodiesel samples from *S. molesta* tend to present better oxidative stability than the commercial sample, as seen in Table 2.

**Table 2.** Analysis of the unsaturation parameters.

| Parameters | CB | EB | MB |
|---|---|---|---|
| MUFA/PUFA | $0.371 \pm 0.002^{c}$ | $0.803 \pm 0.001^{b}$ | $0.823 \pm 0.001^{a}$ |
| LU | $17.65 \pm 0.07^{a}$ | $17.38 \pm 0.09^{b}$ | $17.37 \pm 0.14^{b}$ |
| MLD | $1.41 \pm 0.01^{a}$ | $1.15 \pm 0.01^{b}$ | $1.14 \pm 0.01^{b}$ |

CB = commercial biodiesel; MB = methyl biodiesel; EB = ethyl biodiesel; MUFA = monounsaturated compounds; PUFA = polyunsaturated compounds; LU = average chain length; MLD = average double bonds. Different letters on the line indicate a significant difference ($p < 0.05$) by Tukey's test. Source: The authors.

The lower stability of PUFA is associated with the presence of π-bonds, which are susceptible to oxidative reactions resulting in the degradation of the sample (Antunes Junior, Silva, Carvalho, & Pereira, 2017).

The lower unsaturation level of the produced samples (EB and MB) compared to the commercial (CB) can also be observed in other parameters, with the higher average number of double bonds (MLD) being for the commercial sample (Table 2).

Although the chemical composition was similar in the two production routes, the unsaturation parameters slightly varied, with the methyl biodiesel (MB) showing a higher MUFA/PUFA ratio (Table 2). The higher contents of polyunsaturated compounds in the ethyl biodiesel explain this difference in the two parameters. However, the average number of double bonds was statistically equal since this value can be related to the experimental error, considering that there was no significant difference.

Fatty acid methyl esters with long carbon chains reduce the ignition delay (Zhang, Pham, Kook, & Masri, 2019), exhibiting higher cetane numbers and cloud points (Pinto et al., 2005). Yet, viscosity increases with increasing carbon chains (Pratas et al., 2010), causing more clogging (Pinto et al., 2005) due to gum formation (Saraf & Thomas, 2007). There is also an increase in NOx emissions (Saraf & Thomas, 2007).

The average number of carbons in the samples produced with *S. molesta* was similar but differed from the commercial sample (Table 2). The values obtained were similar to those compiled in the review by Hoekman et al. (2012), which reported values between 13.40 and 19.10 for different oilseeds used for biodiesel production.

## Conclusion

The analysis by attenuated total reflectance-Fourier transform infrared spectroscopy indicated absorption peaks typical of biodiesel. Through gas chromatography, it was possible to determine the composition of biofuels.

The biodiesel from *S. molesta* showed no indication of significant variation in chemical composition in the different routes analyzed (methyl and ethyl), containing a lower content of unsaturated compounds, compared to the commercial sample used as reference.

The biodiesel obtained by the ethyl route showed a higher yield and has potential for use due to its low unsaturation. Nevertheless, it is necessary to optimize the reaction for higher yields.

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