

## Full Length Research Paper

## Characterization of biodiesel obtained from atemoya (*Annona squamosa* × *A. cherimola*) seed oil

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Biodiesel is derived from renewable sources, such as vegetable oils, by means of a transesterification process in which triacylglycerols are transformed into smaller molecules of esters of fatty acids and glycerol. The transesterification reactions of 'Gefner' atemoya (*Annona squamosa* × *A. cherimola*) seed oil extracted by pressing (physical) and solvent (chemical) processes were studied, with analysis of the methyl esters produced. The reactions were monitored using gas chromatography coupled to mass spectrometry (GC-MS), as well as by hydrogen nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR). The methyl esters formed during the transesterification reaction with methanol were determined for each oil. The major methyl esters (16:0, 18:0, 18:1 and 18:2) formed during 50 min of reaction were similar to those reported in the literature for other biodiesels; the peak areas and retention times were also similar. No changes in signal intensity over time were observed for the oils obtained by the two extraction methods. It was also noted that the extraction method had no influence on the types of methyl esters formed during biodiesel production.

**Key words:** Annona, oil extraction, transesterification.

### INTRODUCTION

Biodiesel consists of mono-alkyl esters of long-chain fatty acids derived from renewable sources such as vegetable oils, obtained by a transesterification process in which triglycerides are transformed into smaller molecules of fatty acid esters and glycerol. Its use is intended to replace fossil fuels in diesel engines. It has promising

potential, not only for its important contribution to reducing environmental pollution, but also for the generation of renewable energy as a replacement for fossil diesel and other petroleum products (Pinto et al., 2005).

In 2013, Brazil was the world's second largest biodiesel

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consumer, only behind the United States, which had a demand of 5.2 million m<sup>3</sup>. In terms of production, the USA is the global leader, with production of 5.1 million m<sup>3</sup> in 2013, followed by Germany and Brazil, with production of 3.6 and 3.0 million m<sup>3</sup> of biodiesel, respectively (Agência Nacional do Petróleo (ANP), 2013).

In 2004, the Brazilian government launched the National Program for Biodiesel Production (PNPB). Biodiesel can be used to partially or totally replace mineral diesel for light vehicles, trucks, tractors, and generators. In Brazil, the biodiesel mixture has been regulated by law since 2008. At first, the mandatory mixture was 2%, and it has been progressively increased to 5% (Kohlhepp, 2010).

Biodiesel is registered by the United States Environmental Protection Agency as a fuel and as an additive for fuels (Ferrari et al., 2005). After transesterification, biodiesel can be used neat at 100% (B100) or at proportions of 5% upwards in mixtures whose use is intended to replace fossil fuels in diesel cycle engines, without any need for modification of the engine. Various vegetable oils have been successfully tested in transesterifications with methanol or ethanol for the production of biodiesel. The seeds of peanuts, sunflowers, and soybeans, with oil contents of 41.3, 60.2, and 24.5 g 100 g<sup>-1</sup>, respectively, are widely used for biodiesel production (Constantino et al., 2014). Oils extracted from different fruits have also been explored for biodiesel production (Adekunle et al., 2016; Alexandre et al., 2015), offering non-conventional sources of this biofuel.

Atemoya is an interspecific hybrid of cherimoya (*Annona cherimola*) and sugar-apple (*Annona squamosa*). It was introduced to Brazil in the 1980s and is mainly grown in the south and southeast of the country. In the 1990s, the 'Gefner' hybrid variety was successfully introduced in the northeast of Brazil. The cultivated area now exceeds 1,500 hectares, spread over the States of São Paulo and Paraná, as well as the northeast region (Braga-Sobrinho, 2014).

Atemoya seeds represent around 8.4% of the weight of the fruit and have potential as a source of biodiesel, since the lipid content is 27.3 g 100 g<sup>-1</sup> (Cruz et al., 2013). This content is close to that of other seeds such as soybeans, which are widely used for biofuel. The use of atemoya seeds to produce biodiesel can add value to the fruit.

The objective of this study was to analyze methyl esters produced during the transesterification reaction of the oil from 'Gefner' atemoya seeds, obtained by physical (pressing) and chemical (solvent) extraction.

## MATERIALS AND METHODS

The atemoya was obtained during the 2010/2011 agricultural cycle in an orchard situated in the municipality of Jaíba, in northern Minas Gerais State, Brazil (14°33'-15°28'S, 43°29'-44°06'W, altitude of 500 m). The fruits were harvested at the appropriate stage of

maturity and transported overland to Universidade Federal de Lavras. In the laboratory, the fruits were selected considering size, maturity, and absence of defects. Each replicate employed 82 fruits, totaling 902 fruits.

The seeds were separated and washed with distilled water, weighed, and dried in a forced-air circulation oven at 60 to 65°C until they reached humidity lower than 6%. The seeds were then vacuum-packed in plastic bags and stored at around -10°C in a cold chamber until oil extraction (AOAC, 2012).

### Oil extractions

Oil extractions were performed by pressing (physical) and solvent (chemical) methods, as described by Cruz et al. (2015). Oil pressing was performed in a continuous expeller press, while chemical extraction employed a Soxhlet extractor with hexane as solvent at 68°C. Humidity determination was performed by dehydration of the oil until constant weight in an oven at 105°C, (Lutz, 2008).

### Biodiesel production

Transesterification reactions were performed for 40 min at 50°C in a jacketed reactor, to which 200 ml of vegetable oil and 50 ml of methanol were added. This mixture was heated to a temperature of 50°C under mechanical stirring for 20min. After this time, 6 ml of sodium methoxide (30%) were added, maintaining the temperature and stirring for 40min. The solution was then transferred to a separation funnel for separation of the phases (biodiesel and glycerin) (Silva, 2005). Aliquots were removed at 0, 10, 20, 30, 40, and 50min of reaction. Subsequently, 1ml of each of the six aliquots was treated with 5ml of chloroform, 0.5ml of sulfuric acid, and 10ml of saturated sodium chloride solution. The organic phase obtained was dried with magnesium sulfate, the solvent was removed in a rotary evaporator, and the product was dried with a flow of nitrogen gas. The samples obtained were analyzed using gas chromatography-mass spectrometry (GC-MS), as well as by hydrogen nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR). For the GC-MS analyses, the samples were resuspended in 0.1 ml of hexane.

### Chromatographic analysis

The samples were analyzed using a gas chromatograph coupled to a GC-MS QP2010 Plus mass spectrometer (Shimadzu, Japan) equipped with an AOC-5000 autosampler for liquids and gases (Shimadzu, Japan). A 30 m × 0.25 mm × 0.25 μm RTX-5MS column (5% phenyl to 95% dimethylsiloxane) was used for separation and identification of the compounds. The injector was operated at 220°C in split mode, with a split ratio of 1:20. The carrier gas used was He 5.0, at a flow rate of 1.18 ml min<sup>-1</sup>. The oven temperature was programmed from 60 to 240°C, with a heating ramp of 5°C min<sup>-1</sup>, and then from 240 to 270°C, with a heating ramp of 10°C min<sup>-1</sup>, followed by a final hold at 270°C for 7min. An electron impact mass spectrometer (70 eV) was used in scan mode (45 to 500 Da), with solvent cutting at 3.5min. The detector interface and ion source temperatures were kept at 240°C and 200°C, respectively. The compounds were identified by comparing the mass spectra with library spectra (Wiley 8 and FFNSC 1.2 libraries).

### Nuclear magnetic resonance analysis

The <sup>1</sup>H-NMR analyses employed an EFT-60 spectrometer (Anasazi Instruments, Indianapolis, USA), with one-dimensional spectra acquired for the biodiesel samples obtained by both methods.

**Table 1.** Methyl esters obtained from transesterification of the seed oil from 'Gefner' atemoya (% peak area), using two oil extraction methods: physical (P) and chemical (C).

Methyl esters of fatty acids	Number of carbons from the fatty acid: unsaturation number	Retention time (1 <sup>st</sup> identification)	Reaction time (min)											
			1		10		20		30		40		50	
			P	C	P	C	P	C	P	C	P	C	P	C
Methyl octadecanoate	18:0	24.45	25.46	24.65	19.95	20.74	18.50	21.31	20.95	19.96	22.85	21.86	20.64	23.42
Methyl nonanoate	9:0	26.57	0.09	-	-	-	0.02	-	-	-	-	-	0.04	-
Methyl eicosenoate	20:0	28.10	0.07	8.95	0.08	-	0.12	3.5	3.05	2.83	0.03	-	0.06	-
Methyl hexadecanoate	16:0	28.62	27.39	29.39	21.26	21.49	20.47	22.85	22.72	21.61	24.54	23.85	22.2	24.30
Methyl (9z)-9-octadecenoate	18:1	30.09	41.95	24.31	56.62	29.96	32.89	27.95	29.2	29.82	25.06	26.13	28.69	25.26
Methyl heptadecanoate	17:0	30.53	0.64	1.77	0.66	0.47	0.39	0.64	0.62	0.44	0.87	0.64	0.44	-
Methyl nonadecanoate	19:0	34.16	0.14	0.61	0.14	-	-	-	-	-	0.19	0.14	-	-
Methyl eicosanoate	21:0	35.86	3.25	-	-	2.92	1.76	-	-	-	4.58	3.78	2.96	6.25
Methyl docosanoate	10:0	38.62	0.56	1.64	0.48	0.49	0.25	0.47	0.51	0.48	0.79	0.58	0.51	0.98
Methyl tetracosanoate	24:0	41.04	0.34	0.9	0.31	-	0.16	0.34	0.32	0.31	0.47	0.29	0.3	0.51
Methyl tetradecanoate	14:0	24.45	-	0.49	0.19	-	0.06	0.24	0.19	0.16	0.3	-	-	-
Methyl (9z)-9-hexadecenoate	16:1	28.19	-	1.31	0.3	0.45	0.3	0.94	-	0.36	1.56	0.94	0.5	1.65
Methyl (9z,12z)-9,12-octadecadienoate	18:2	31.86	-	3.73	-	23.08	24.86	21.12	22.04	23	17.72	21.03	22.81	15.32
Methyl (11e)-11-eicosenoate	20:1	35.45	-	1.53	-	-	0.14	-	-	0.44	0.84	0.59	0.42	1.15

Previously treated biodiesel samples (0.1ml) were dissolved in 0.1ml of chloroform deuterated with 99.8% deuterium (CDCl<sub>3</sub>), in 5 mm NMR tubes. Tetramethylsilane (0.1 ml) was used as an internal reference standard.

## RESULTS AND DISCUSSION

The yield of the transesterification reaction for the oil obtained by pressing of atemoya seeds was 89% methyl esters and 11% glycerin. The biodiesel yield for the chemical extraction was 91% methyl esters and 9% glycerin. These values differed from the results obtained for soybean biodiesel by Ferrari et al. (2005), who reported 57.26% ethyl esters, 22.29% glycerin, 10.04% recovered ethanol, and 10.41% losses. The concentration of glycerin in the biodiesel obtained from atemoya seeds was approximately half that obtained from soybeans, so the concentration of

biodiesel was much higher, and losses were not observed.

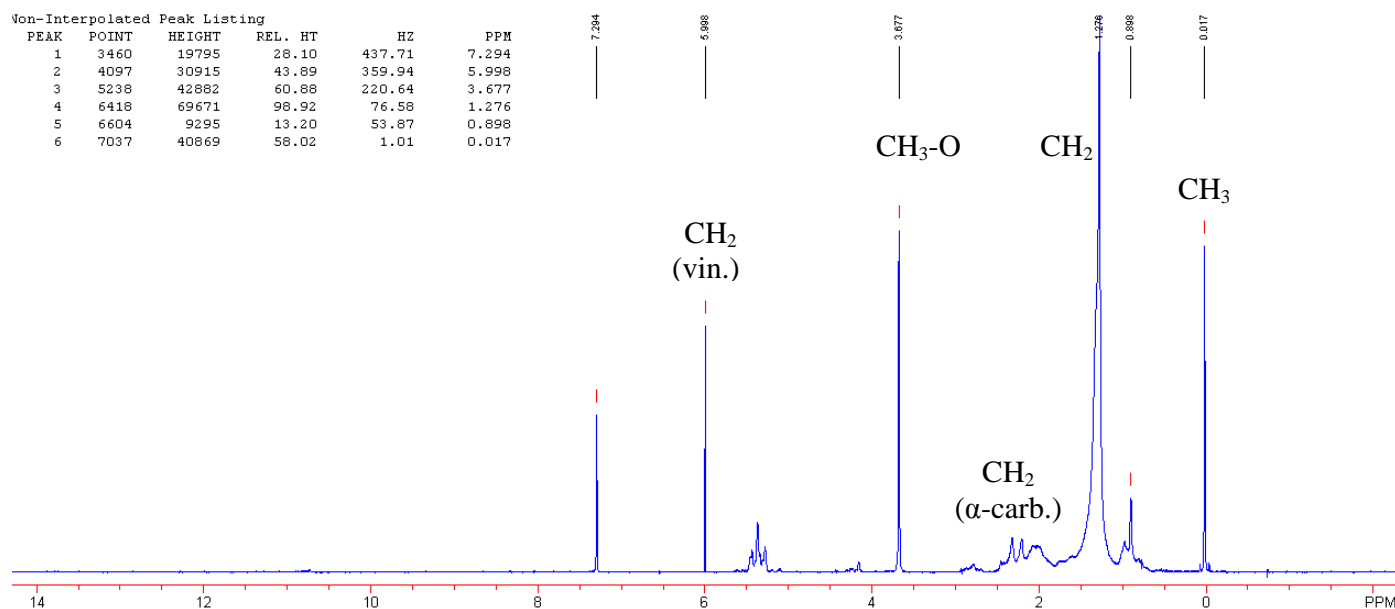
Table 1 shows the profile of methyl esters for the transesterification reaction of the oil from 'Gefner' atemoya seeds. The major esters found in the atemoya biodiesel were 16:0, 18:0, 18:1, and 18:2.

The ester area percentages obtained for the physical and chemical extractions, after a reaction time of 50min, at which the biodiesel was decanted, were 22.2 and 24.3 (16:0), 20.64 and 23.42 (18:0), 28.7 and 25.26 (18:1), and 22.81 and 15.32 (18:2), respectively. Comparison of the results obtained here with the findings of Urioste et al. (2008) revealed the presence of one additional ester (18:2).

In chromatographic, determination of the esters of fatty acids was obtained in transesterification reactions of babassu oil with ethanol, propanol,

and butanol. Urioste et al. (2008) reported the following areas (%) for ethyl, propyl, and butyl esters, respectively: 24.82, 22.82, and 23.52 (16:0); 21.56, 22.14, and 23.28 (18:0); and 24.60, 22.50, and 24.56 (18:1). It appears that there was virtually no difference between the areas of the three ethyl, propyl and butyl esters formed in babassu biodiesel.

In their study with soybean oil, Ferrari et al. (2005) observed the following areas (%) for ethyl esters of fatty acids: 11.29 (16:0), 3.54 (18:0), 22.45 (18:1), 54.62(18:2), and 8.11 (18:3), with a predominance of unsaturated fatty acids. These esters were the same as those obtained in the transesterification of atemoya seed oil, with the exception of the 18:3 ester, which was not found in atemoya oil. The 18:2 methyl ester of the atemoya seed oil extracted by the pressing and solvent methods showed areas of 22.81% and



**Figure 1.**  $^1\text{H-NMR}$  spectrum of methyl esters obtained in the transesterification of the 'Gefner' atemoya seed oil extracted physically (by pressing), at 50 min reaction time.

15.32%, respectively, which were smaller than the value obtained for soybean oil. On the other hand, the 18:0 and 18:1 esters of atemoya oil had greater areas, compared to the soybean oil, with values of 20.64% (pressed) and 23.42% (solvent extracted), and 28.69% (pressed) and 25.26% (solvent extracted), respectively. These data show that the predominant esters in current biodiesels are 16:0, 18:0, 18:1, 18:2, and 18:3.

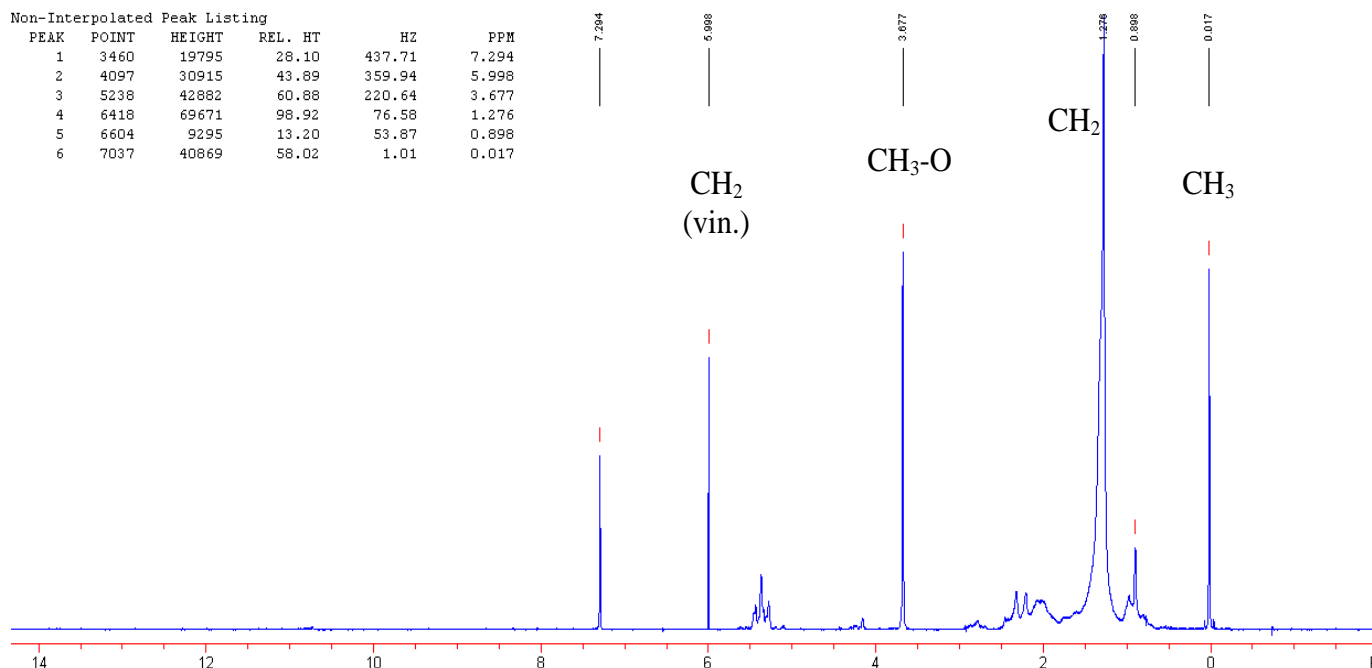
Marques et al. (2010) emphasized the importance of considering instrumental precision when evaluating major peak areas, in order to increase the accuracy of determination of esters of fatty acids formed during transesterification reactions. Barbosa et al. (2010) reported the following areas (%) for ethyl esters of soybean seed oil: 16.0 (16:0), 2.4 (18:0), 23.5 (18:1), and 51.2 (18:2). In the present work, greater areas were obtained for 16:0, 18:0, and 18:1 esters in the atemoya oil biodiesel obtained using both forms of oil extraction (press and solvent), while the value obtained for the 18:2 ester was smaller. Benito et al. (2014) studied the biodiesel potential of *Annona diversifolia* seed oil and reported the following areas (%) for methyl esters: 16.4 (16:0), 5.22 (18:0), 70.4 (18:1), and 7.97 (18:2). The areas obtained for the 16:0, 18:0, and 18:2 methyl esters were greater for the decanted biodiesel from atemoya oil extracted by both techniques (physical and chemical), while the area was smaller for the 18:1 ester.

Methanol is the main alcohol used in transesterification in many countries (Pinto et al., 2005). In Brazil, several research groups and small producers use the methyl pathway for the production of biodiesel, because methanol is more reactive, while ethanol causes greater

dispersion of glycerin in the biodiesel, making separation difficult (Lôbo et al., 2009). A reaction time of 30min was required for the formation of methyl esters in the biodiesel, similar to the duration of 25min reported by Urioste et al. (2008) for biodiesel from Babassu, where the major esters formed were 16:0, 18:0, and 18:1.

Encinar et al. (2002) observed that the transesterification reaction was very fast, with conversion into ethyl esters close to the maximum value after only 5 to 10min of reaction, and stabilization at a maximum value after 20 to 30min. These values were similar to those found for the formation of biodiesel from atemoya oil, which occurred after 20 to 30min of reaction. However, in the study of Ferrari et al. (2005), chromatographic monitoring of the products formed after various reaction times showed that a time of 5min was sufficient for the conversion of neutral and dried oil into ester. The conversion of fatty acids into methyl esters in the atemoya oil occurred between 5 and 10min of reaction, stabilizing at a maximum value after 20 to 30min. The physical extraction (pressing) of atemoya seed oil is economically advantageous and provides a high oil extraction efficiency of  $88.9\text{g } 100\text{g}^{-1}$  (dry mass basis) (Cruz et al., 2015).

Furthermore, in comparison with chemical extraction using solvent, a disadvantage of the latter has greater oxidation of the extracted oil. The profile of the methyl esters identified by GC-MS was confirmed by  $^1\text{H-NMR}$ . Figures 1 and 2 show the major chemical shifts characterizing the esters, formed after 50 min of reaction. No changes in signal strength or in the hydrogen chemical shifts with time were observed after the



**Figure 2.**  $^1\text{H-NMR}$  spectrum of methyl esters obtained in the transesterification of the 'Gefner' atemoya seed oil extracted chemically (using solvent), at 50 min reaction time.

**Table 2.** Chemical shifts (ppm) obtained by  $^1\text{H-NMR}$  (60 MHz) of biodiesel in  $\text{CDCl}_3$  at  $25^\circ\text{C}$  (physical extraction).

H	$\delta_{\text{H}}$	
	Atemoya biodiesel	Paiva et al. (2010)
$\text{CH}_3$	0.89	0.90
$\text{CH}_2$	1.27	1.2 – 2.5
$\text{CH}_2$ ( $\alpha$ -carbonyl)	2.40	2.1 – 2.5
$\text{CH}_3\text{-O}$	3.68	3.5 – 4.8
$\text{CH}_2$ (vinylic)	5.99	4.5 – 7.0

**Table 3.** Chemical shifts (ppm) obtained by  $^1\text{H-NMR}$  (60 MHz) of biodiesel in  $\text{CDCl}_3$  at  $25^\circ\text{C}$  (chemical extraction).

H	$\delta_{\text{H}}$	
	Atemoya biodiesel	Paiva et al. (2010)
$\text{CH}_3$	0.89	0.90
$\text{CH}_2$	1.27	1.2 – 2.5
$\text{CH}_2$ ( $\alpha$ -carbonyl)	2.40	2.1 – 2.5
$\text{CH}_3\text{-O}$	3.68	3.5 – 4.8
$\text{CH}_2$ (vinylic)	5.99	4.5 – 7.0

transesterification reactions of the atemoya oils obtained by the two extraction methods (physical and chemical). This suggests that the extraction method had no influence on the characteristics of the oil obtained, or, therefore, on the product (biodiesel). Tables 2 and 3 show the  $^1\text{H-NMR}$  data (60 MHz) for the biodiesel (in  $\text{CDCl}_3$ ), compared with the literature. There was a good correlation of the chemical shifts in the spectra with the data reported by Paiva et al. (2010).

## Conclusions

The methyl esters formed from the 16:0, 18:0, 18:1, and 18:2 fatty acids during the transesterification reaction were similar to those present in other current biodiesels.

The method used for oil extraction had no influence on the types of methyl esters formed during biodiesel production.

The oil from atemoya seeds has potential for use as biodiesel, with advantages including addition of value to the fruit and a use for unwanted biomass (the seeds) that might otherwise be treated as waste.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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