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Chemical and physicochemical characterization of the seed oil from 'Gefner' atemoya

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Atemoya is a hybrid fruit derived from the crossing of sugar-apple (*Annona squamosa* L.), with cherimoya (*Annona cherimola* Mill.). The objective of this study was to compare two extraction methodologies for the seed oil from 'Gefner' atemoya, for yield, chemical and physicochemical characteristics, in order to use the oil as biofuel. The physical extraction was shown to be more economically viable than the chemical, presenting a lower amount of residues and not leading to the oxidation of the extracted oil. Saponification and ester values were high, showing that the oils have a high triacylglycerol content. Fatty acid profile, iodine value and refractive index confirmed the high degree of unsaturation of the fatty acids, demonstrating the advantage of ensuring high fluidity. The oil presents a high acidity, requiring a neutralization process. The different extraction techniques did not significantly interfere in the analyzed values, except for peroxide value. The major fatty acids are the oleic, palmitic and stearic. 'Gefner' atemoya seed represents a source of good quality oil, and could be safely applied in the production of biofuel.

Key words: Annona, biofuel, fatty acids.

INTRODUCTION

Atemoya is a hybrid fruit derived from the crossing of a tropical fruit, sugar-apple, also known as ata (*Annona squamosa* L.), with cherimoya (*Annona cherimola* Mill.). Native to the Andean regions of Chile, Peru, Bolivia, Ecuador and regions of mild climate. Atemoya had its origin in 1908, when the first artificial crossing was performed in the United States Department of Agriculture's Subtropical Laboratory, in Miami. For a long time, there was a certain disinterest in the fruit but, in the 1940s, studies were initiated in Israel, aiming to standardize its propagation (Morton, 2014). 'Gefner' atemoya presents on average 56 seeds per fruit, equivalent to 8.4% of its total weight, and its total lipid content is 27.3% (Cruz, 2013). This content is relatively

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high, when compared to those of other seeds, which are oil sources used as biofuel. Plant seeds are usually discarded by industry and consumers, but could be used as an alternative oil source, and be safely applied in the chemical industry, adding more value to the fruit. There is a lack of information on atemoya seed oil in the research literature. This hybrid can meet desirable and relevant characteristics coming from the two species that participated in its crossing, and it is important to conduct research to develop a technological extraction of the oil from these seeds, which must be efficient, economic and safe to the environment. Therefore, the objective of this study was to compare two extraction methodologies for the seed oil from 'Gefner' atemoya, for yield, chemical and physicochemical characteristics, in order to use the oil as biofuel.

MATERIALS AND METHODS

The orchard where the atemoya was grown is situated in the municipality of Jaíba, located in Northern Minas Gerais, during the 2010/2011 agricultural cycle. The geographical coordinates lie between 14°33' and 15°28' south latitude, and 43°29' and 44°06' west longitude, at 500 m altitude. The average annual temperature is 24°C and the average summer and winter temperatures are 32 and 19.5°C, respectivel with an average annual rainfall of 900 mm. The fruits were harvested at the appropriate maturation stage, when mean slope of the spines, packed in cardboard boxes and sent overland to Lavras, MG, 1000 km away from Jaíba. The refrigerated transport (20°C) took approximately 10 h. In Lavras, the fruits were transferred to the laboratory, where they were selected for the absence of defects, size and maturity stage, with 82 fruits for each replicate, totaling 902 fruits. The seeds from each replicate were separated and washed with distilled water, weighed and dried in a forced-air circulation oven, with temperatures ranging from 60 to 65°C, until they approximately reached a humidity lower than 6%. The seeds were then vacuum-packed in plastic bags and stored at -10°C in a cold chamber until oil extraction, according to the methodology proposed by the AOAC (2012).

Oil extractions were performed by pressing and solvent. Oil pressing was performed in a continuous expeller press. For the oil extraction, 3 kg of dry whole seeds were used. The chemical extraction was performed with a Soxhlet extractor using hexane as solvent (68°C); the oil was then rotaevaporated at 70°C, using vacuum with a reduced pressure of -760 mmHg for 4 h in order to remove the solvent remaining in the sample; no more hexane was removed during this time interval. These oils were collected in amber glass containers and stored at approximately -20°C to prevent uncontrollable oxidative changes, according to the methodology proposed by the AOCS (1998). The oils extracted from the two types of treatment were characterized according to standard methods of analysis for oils and fats from the Adolfo Lutz Institute (2008). Humidity determination was performed by oil dehydration in an oven at 105°C, until constant weight. The determination of total insoluble content consisted in dissolving the residue (resulting from humidity determination) with ether, filtering with filter paper and heating in an oven at 105°C until constant weight. Ash determination was performed by heating the filter paper containing the total insoluble content in ether, incinerating it in a muffle at 550°C for 3 h. The determination of organic insoluble content in ether was performed in order to reduce the weight of the total insoluble content, as well as the weight of the corresponding ash. The determination of total lipids was performed from 100 g sample, subtracting the mass of humidity from the mass of total insoluble content in ether.

The iodine value determines the degree of unsaturation of the oil and was measured by the Wijs method, and the result is expressed in g 100 g^{-1} dry basis (DB). The acid value was determined by the amount of sodium hydroxide used to neutralize the free fatty acids in one gram of oil, and the result is expressed in g 100 g^{-1} DB. The peroxide value indicates the degree of oxidation of an oil, determined by titration with sodium thiosulphate in the presence of a saturated solution of potassium iodide, with a result expressed in meq kg^{-1} DB. The saponification number is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the hydrolysis of 1 g of oil and is expressed in mg KOH g^{-1} DB. The refractive index was determined with an Abbe refractometer at 25°C. The difference between saponification and acid values results in the ester value, which represents the amount of triacylglycerols in the sample, and is expressed in mg KOH g^{-1} DB (Cocks and Van Rede, 1966). For the determination of the fatty acid profile, oil esterification was performed, using the method of Hartman and Lago (1973): 2 mL of 0.5 mol L^{-1} NaOH were added in methanol, to the residue obtained after evaporation. It was then placed in a boiling bath for 5 min, where 2.5 mL of esterifying reagent were added. It was again placed in a boiling bath for 5 min, and then cooled. After cooling, 2.0 mL of saturated NaCl and 2.5 mL of hexane were added. After stirring, the sample was centrifuged at 2,500 x *g* for 10 min. The phases were separated, the bottom was discarded and the upper part was stored for solvent evaporation, using gaseous nitrogen. The obtained residues were stored under refrigeration until the analyses were performed.

The fatty acid composition was determined by gas chromatography, and a chromatograph (GC-2010, Shimadzu) equipped with a mass detector and a 30 m x 0.25 mm fused-silica capillary column was used, containing polyethylene glycol as the stationary liquid phase. The standard used was a mixture of 37 methyl esters (SupelcoTM 37 Component FAME Mix), from C:4 to C22:6, with a purity of 99.9%. The following operating parameters were used: "split" injection mode, with a split ratio of 1:20; injection volume of 1 µL; temperature of detector at 240°C; temperature of injector at 220°C; temperature program: beginning at 60°C with a linear ramp of 5°C/min until reaching 240°C; this temperature was kept for 5 min, as well as the heating ramp, at 10°C/min until reaching 270°C, keeping this temperature. To perform the gas chromatography, it was necessary to redissolve the samples in 0.5 mL hexane. Peak identification was performed by a comparative method with the retention times of the standard fatty acid esters and the results were performed by integration of the peak areas, and expressed in area percentage. The experiment was conducted in a completely randomized design in a 2 x 11 factorial scheme, with two treatments (physical extraction and chemical extraction), and eleven repetitions.

Statistical analyses were performed according to standard techniques of the Sisvar software. When the analysis of variance showed a significant difference, the Tukey test was used to compare means, with a probability of 5%.

RESULTS AND DISCUSSION

The results of the present study were not compared with other seed oils from other Annonaceae, since records were not found in the literature. The oil yield in the chemical extraction was 25.92 ± 0.53 g 100 g^{-1}, higher than that obtained by pressing, which was 20.04 ± 2.11 g 100 g^{-1}. Freire (2001) determined the yield of castor seed oil by chemical extraction and found 48.6 g 100 g^{-1}. The yield of atemoya seed oil was lower than that of castor bean; however, it is still quite high. The physical

Table 1. Chemical composition of the seed oil from 'Gefner' atemoya, from two types of extraction.

Chemical component	Extractions physical	Chemical
Total lipids (g 100 g^{-1} dry basis - DB)	95.6 ± 0.2a	88.7 ± 0.3b
Humidity (g 100 g^{-1})	0.9 ± 0.1a	7.6 ± 0.1b
Total insolubles (g 100 g^{-1} DB)	3.5 ± 0.1	3.5 ± 0.3
Organic insolubles (g 100 g^{-1} DB)	3.2 ± 0.1	3.0 ± 0.3
Ash (g 100 g^{-1} DB)	0.3 ± 0.0b	0.5a ± 0.1

Data are the mean of 11 replicates ± standard deviation. Different letters indicate significant differences by the Tukey test at 5% probability.

Table 2. Physicochemical characteristics of the seed oil from 'Gefner' atemoya, from two types of extraction.

Parameter	Type of extraction	
	Physical	Chemical
Iodine value (g 100 g^{-1} dry basis - DB)	82.0 ± 0.29	82.4 ± 0.52
Acid value (mg KOH g^{-1} DB)	1.37 ± 0.29	1.42 ± 0.05
Peroxide value (meq kg^{-1} DB)	11.1 ± 0.0b	22.6 ± 0.0a
Saponification number (mg KOH g^{-1} DB)	152.93 ± 0.12b	160.47 ± 2.30a
Refractive index (nD)	1.170 ± 0.0a	1.136± 0.01b
Ester value (mg KOH g^{-1} DB)	151.56 ± 0.35	159.05 ± 2.35
Ester (%DB)	99.10 ± 0.16	99.12 ± 0.05

Data are the mean of 11 replicates ± standard deviation. Different letters indicate significant differences by the Tukey test at 5% probability.

extraction has the advantage of being economically viable, leading to a higher total lipid content, with 95.6 g 100 g^{-1} DB, whereas in the chemical extraction, the content was lower, with 88.9 g 100 g^{-1} DB (Table 1). Furthermore, the chemical extraction has the disadvantage of the presence of hexane (approximately 6.7 g 100 g^{-1} DB remained in the oil), since the rotaevaporation process did not completely remove hexane, and resulted in a higher oxidation of the extracted oil, shown later in the analysis of the peroxide value. Oven drying at 65°C represented a good form of seed dehydration which, after drying, exhibited 3.3 g 100 g^{-1} humidity. The oil from the physical extraction had 0.9 g 100 g^{-1} humidity, and this content is not a problem, since, for its use, this content may be reduced to the ideal value, which is 0.5 g 100 g^{-1} according to ANP (2010), performing a dehydration in an oven. The levels of total insoluble content and organic insoluble content in ether were statistically equal, showing that the extraction method did not influence the level of these constituents. The levels of organic insoluble content in ether were lower than those reported by Oliveira et al. (2011), who conducted a study with pequi oil and found 5.95 g 100 g^{-1} DB. The ash content was 0.5 g 100 g^{-1} DB for the chemical extraction, higher than that of the physical extraction, which was 0.3 g 100 g^{-1} DB.

According to Lutz (2008), organic insoluble content in ether indicates the amount of insoluble matter in the organic compound and the amount of residue resulting from the extraction, reducing the quality of the oil and increasing the possibility of rancidification. The results for the physicochemical characteristics of atemoya seed oils are shown in Table 2. The iodine value (II) of the seed oil from 'Gefner' atemoya in the physical and chemical extractions was statistically equal, 82.0 g 100 g^{-1} DB and 82.4 g 100 g^{-1} DB, respectively. These contents were higher than those by Oliveira et al. (2011), who conducted a study with pequi oil and found 6.2 g 100 g^{-1} DB; and smaller than that of castor seed oil (BRS-149) reported by Costa (2006), which was 92.27 g 100 g^{-1} DB. Atemoya seed oil meets the recommendation of ANVISA due to its higher fluidity, satisfying an important characteristic required for use as a biofuel. The Brazilian legislation (Anvisa, 2005) places no limits for II in vegetable oils for use in biofuels but, according to the AOCS (1998), the optimal II must be between 81 and 91 g 100 g^{-1}. The acid value (IA) in the physical and chemical extractions was also statistically equal, 1.37 g 100 g^{-1} DB and 1.42 g 100 g^{-1} DB, respectively. This IA is fairly high, compared with the specifications established by the National Petroleum Agency (ANP, 2010) for vegetable oils, which must be lower than 0.5 mg KOH g^{-1} DB. The obtained values would be appropriate for the production of biodiesel, since they are fixed in the transesterification reaction.

Silva (2005) found an IA of 1.87 g 100 g^{-1} DB in andiroba

oil, a value which was higher than that found for atemoya seed oil. According to Canakci and Van Gerpen (2001), a high acidity can be neutralized with a basic catalyst in a transesterification, and a higher amount of catalyst would be required for the reaction to be conducted efficiently. The peroxide value (IP) in the physical extraction (11.1 meq kg^{-1} DB) was statistically different from that obtained for the chemical extraction (22.6 meq kg^{-1} DB). Only the IP of the oil from the physical extraction is close to the recommended since, according to Anvisa (2005), the IP should be a maximum of 10 meq kg^{-1} DB. According to Lutz (2008), the amount of peroxide indicates the extent to which the oxidation progressed. There was a significant difference between the saponification numbers (IS) for the two extractions, with 152.93 mg 100 g^{-1} DB for the physical and 160.47 mg 100 g^{-1} DB for the chemical. These numbers were lower than those found by Silva (2005), who found 193.84 mg 100 g^{-1} DB for *Carapa guianensis* Aubi., oil. According to the AOCS (1998), the ideal is that the IS is between 176 and 187 mg g^{-1}. The values in atemoya seed oil were just below the recommended for biofuel production, which has fatty acids with a low molecular weight.

For the refractive index (IR), there was no significant difference for the two extractions, with 1.170 for the physical extraction and 1.136 nD for the chemical, and these values were considered low, when compared with that of *Ricinus communis* L. (Cultivar BR-188 Paraguaçu), which was 1.466 nD, according to Costa (2006). According to the AOCS (1998), the ideal is that IR values range between 1.473 and 1.4773 nD. The IR of oils and fats is often used as a criterion of quality and identity since, for oil, this index increases with II and can be used in the control of the hydrogenation of unsaturated oils (Cecchi, 2003). The ester value (IE) allows calculating the percentage of ester which represents the amount of triacylglycerols present in the oil. There was no significant difference between the IE for the extractions, and the percentage of ester for the two extractions was almost insignificant, with 151.56 mg KOH g^{-1} DB and 99.10% DB for the physical extraction and 159.05 mg KOH g^{-1} DB and 99 12% DB for the chemical extraction. These values are considered as excellent for the application in biofuel since, according to the ANP (2010), the percentage of ester must be at least 96.5%.

Table 3 presents the fatty acid composition of the seed oil from 'Gefner' atemoya for two types of extractions. Unsaturated fatty acids of oils resulting from physical (66.5%) and chemical (66.7%) extractions were in a higher amount. Atemoya seed oil showed a lower content of unsaturated fatty acids, compared with araticum pulp oil (79.3%), and a higher content than that of the *Butia capitata* oil (63.8%) (Lopes et al., 2012).

The highest percentage of unsaturated fatty acids, for the physical and chemical extraction, respectively, was recorded for 9-octadecenoic acid ($18:\Delta^{9}$), known as oleic acid, with 58.8 and 46.8%, followed by 11-eicosanoic acid ($20:\Delta^{11}$), known as vaccemic acid, with 6.6 and 6.5%; and 9-hexadecenoic acid ($16:\Delta^{9}$), with 0.7 and 0.7%, known as palmitoleic acid. Lopes et al. (2012) found 66% of oleic acid; 0.24% of vaccemic acid and 0.23% of palmitoleic acid in *Annona coriacea* pulp oil, therefore, with percentages different from the results of this study. Unsaturated fatty acids with the lowest percentage were cyclopropane-octanoic acid (8:0), with 0.2 and 0.2%, and 9,12-octadecadienoic acid ($18:\Delta^{9,12}$), known as linoleic acid, with 0.2 and 0.2%.

Lopes et al. (2012) found higher contents of linoleic acid (1.55%) in the araticum oil. The percentage of saturated fatty acids was 33.5 and 34.2%, resulting from the physical and chemical extraction, respectively. Lopes et al. (2012) reported a percentage of unsaturated fatty acids of 29.7% in araticum pulp oil, lower than that of this study. The major saturated fatty acids in atemoya seed oil, resulting from chemical and physical extractions, respectively, were hexadecanoic acid (16:0), with 15.0 and 13.4%, known as palmitic acid; octadecenoic acid (18:0), with 14.1 and 14.5%, known as stearic acid. Lopes et al. (2012) reported, in the araticum oil, 10.78% palmitic acid, followed by 6.83% stearic acid, as major saturated acids.

The contents of oleic acid were similar to those of Segall et al. (2006) and Lima et al. (2007) in *Caryocar brasiliense* pulp oil. Oleic (48.7 to 57.4%) and palmitic (34.4 to 46.79%) acid were identified, and the contents of palmitic acid were higher than those found in atemoya. They also reported, in descending order, the following acids: palmitoleic, linoleic, linolenic, stearic and arachidic, among others, and in atemoya, in descending order, are the following acids: oleic, palmitic, stearic, vaccemic, palmitoleic and linoleic.

Conclusion

The physical extraction of the seed oil from 'Gefner" atemoya proved to be a good extraction methodology for oil, since it has a smaller amount of resulting residues, keeping oil quality and reducing the possibility of rancidification, being an economically viable process. The fatty acid profile, II, IR, IA, IS and IE, showed values which were considered excellent for use as biodiesel in this study. It is possible to conclude that the use of the seed oil from 'Gefner' atemoya as a raw material is a safe application in the production of biofuel.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Table 3. Fatty acid profile of the seed oil from 'Gefner' atemoya, from two types of extraction, expressed as % of peak area on the chromatogram.

IUPAC name (1979)	Fatty acids	Extractions	
		Physical	Chemical
Pentanoic acid	5:0	traces	0.2 ± 0.0
Tetradecanoic acid	14:0	0.2 ± 0.0	0.2 ± 0.0
Pentadecanoic acid	15:0	traces	0.1 ± 0.1
Hexadecanoic acid	16: 0	15.0 ± 0.7	13.4 ± 3.5
Heptadecanoic acid	17:0	0.8 ± 0.2	0.8 ± 0.2
Octadecanoic acid	18:0	14.1 ± 0.4	14.5 ± 3.7
Nonadecanoic acid	19:0	0.3 ± 0.1	0.3 ± 0.1
Heneicosanoic acid	20:0	traces	0.1 ± 0.0
Docosanoic acid	22:0	1.2 ± 0.1	1.4 ± 0.7
Tricosanoic acid	23:0	0.2 ± 0.0	0.2 ± 0.1
Tetracosanoic acid	24:0	1.1 ± 0.1	1.4 ± 0.9
Pentacosanoic acid	25:0	0.2 ± 0.0	0.1 ± 0.1
Hexacosanoic acid	26:0	0.2 ± 0.0	0.1 ± 0.0
Cyclopropane-octanoic acid	8:0	0.2 ± 0.0	0.8 ± 0.1
Benzoic acid	$7:3(\Delta^{1,3,5)}$	nd	0.3 ± 0.0
7-hydroxy-7-methyl-octanoic acid	$8:2(\Delta^{7\text{-OH-7ME}})$	nd	0.1 ± 0.1
9-hexadecenoic acid	$16:1(\Delta^{9})$	0.7 ± 0.2	0.7 ± 0.2
11-eicosanoic acid	$20:1(\Delta^{11})$	6.6 ± 0.2	6.5 ± 0.5
9-octadecenoic acid	$18:1(\Delta^{9})$	58.8 ± 0.4	46.8 ± 1.1
10-octadecenoic acid	$18:1(\Delta^{10})$	nd	11.6 ± 8.3
9,12-octadecadienoic acid	$18:2(\Delta^{9,12})$	0.2 ± 0.0	0.1 ± 0.0
ΣSaturated		33.5 ± 4.2	34.2 ± 4.0
ΣUnsaturated		66.5 ± 12.8	66.7 ± 10.4

Data are the mean of 11 replicates ± standard deviation. nd, not detected.

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