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Fatty acid composition and some physicochemical characteristics of *Sterculia apetala* seed oils

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SUMMARY: In the tropical rain forests of southeastern Mexico, the use of *Sterculia mexicana* and *Sterculia apetala* seed oils for human and animal nutrition is common. However, the seeds contain cyclopropene fatty acids, whose consumption is related with beneficial as well as detrimental physiological effects. The aim of this study was to determine the fatty acid profile and the physicochemical characteristics of *S. apetala* seed oil and to evaluate the effect of roasting on both aspects. Cyclopropenoic fatty acids, sterculic acid and malvalic acid were identified in the natural and roasted seed oils. The major component in the seed oil was sterculic acid, as has been reported for *Sterculia mexicana* and *Sterculia foetida*. The roasting process modified some physicochemical properties and the fatty acid composition of the seed oil, particularly by decreasing its content of sterculic acid. To our knowledge, this is the first report on the fatty acid composition of *S. apetala* seed oil.

KEYWORDS: *Cyclopropenoic Fatty Acids; Sterculia apetala; Sterculia mexicana; Sterculic acid*

RESUMEN: ***Determinación de la composición de ácidos grasos y algunas características fisicoquímicas del aceite de semillas de Sterculia apetala***. En zonas tropicales del sureste de México, el uso de semillas de *Sterculia mexicana* y *Sterculia apetala* es común para consumo humano y animal. Sin embargo, dichas semillas contienen ácidos grasos ciclopropenoicos, los cuales se les ha relacionado tanto con efectos fisiológicos beneficiosos como adversos para la salud. El objetivo de este estudio fue determinar el perfil de ácidos grasos y las características fisicoquímicas de la especie *S. apetala*, así como la evaluación del aceite sometido a un proceso de tostado. Se identificaron ácidos grasos ciclopropenoicos como el ácido estercúlico y malválico, en el aceite natural y tostado. Para las especies *S. mexicana* y *S. foetida*, el componente mayoritario en las semillas fue el ácido estercúlico. El proceso de tostado modificó algunas propiedades fisicoquímicas y la composción de los ácidos grasos, especificamente disminuyó el contenido de ácido estercúlico. Para nuestro conocimiento, este es la primera información publicada sobre la composición de los ácidos grasos de la especie *S. apetala*.

PALABRAS CLAVE: *Ácido estercúlico; Ácidos grasos ciclopropenoicos; Sterculia apetala; Sterculia mexicana*

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1. INTRODUCTION

The genus *Sterculia* comprises 300 species of trees that belong to the order Malvales, occurring mostly in tropical regions of the world. The most extensively studied among them is *Sterculia foetida* L., which is found from Eastern tropical Africa to North Australia (Mujumdar *et al*., 2000). Its leaves, roots and gum are used in herbal medicine as aperient, diuretic, anti-inflammatory, and as a central nervous system depressant (Chopra *et al*., 1992).

The species *Sterculia mexicana* (R. Br), locally known as "Castaño", and *Sterculia apetala* (Jacq.), locally known as "Bellota", "Castaña" or "Tepetaca", are found in the tropical rain forests of southeastern Mexico in the states of Veracruz, Tabasco, Oaxaca and Chiapas (Penington and Sarukhán, 2005). They are exotic deciduous trees, 30–40 m in height, with horizontal branches grouped in the top of the tree in *S. mexicana*, or distributed over the tree trunk in *S. apetala*. The leaves are palmated-lobulated, 15–50 cm long, and located in groups at the end of the branches. The flowers are yellow with red or purple stripes. The fruits are brown (*S. apetala*) or redish (*S. mexicana*) follicles, 8–10 cm long, found in groups of 3–5, that contain 10–15 black seeds of 2.5×1.5 cm, each covered by orange stinging spicules. The seeds are boiled or roasted (to develop a nutlike or peanut butter-like aroma and taste) and used for human nutrition and as animal forage. In some areas, grounded seeds are used for chocolate flavoring (Benitez *et al*., 2004; Vazquez-Torres *et al*., 2010).

It has been reported that the oils extracted from plants from the order Malvales, Fabales and Sapindales naturally contain cyclopropene fatty acids (CPFA) which are unusual fatty acids containing a three member carbocyclic ring forming the center of their 18 and 17 carbon chains (Bao *et al*., 2003; Vickery, 1980). Sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid] (Figure 1A) and malvalic acid [7-(2-octyl-1-cyclopropenyl) heptanoic acid] (Figure 1B) are CPFA that contain a highly strained and reactive cyclopropene ring in their carbon chains. They are present in variable amounts in these plants, the highest content being reported for *Sterculia foetida* seeds oil, where the sterculic acid content is around 55% (Corl *et al*., 2001). In addition to its role as energy and carbon storage in seeds, it has been proposed that it may serve as a protective agent against fungal and insect attack (Schimid and Patterson, 1988). Moreover, various biological activities have been observed after sterculic acid administration in cell cultures and animal models, showing beneficial as well as adverse results. Some studies have demonstrated that sterculic acid provokes reductions in body weight and adiposity, improvements in glucose tolerance and attenuates hepatic inflammation (Gomez *et al*., 2003; Major *et al*., 2008; Ortinau *et al*., 2012), while others have suggested adverse effects on smooth muscle proliferation, reproductive function and growth, induction of hypercholesterolemia and liver damage, and carcinogenic effects when given at high doses to rats (>1%) (Mujumdar *et al*., 2000; Major *et al*., 2008; Lee *et al*., 1971; Look *et al*., 2004; Pawlowski *et al*., 1985). Some of these effects have been related to the ability of sterculic acid to inhibit stearoyl–CoA desaturase activity (SCD, also known as Δ9 desaturase), a central lipogenic enzyme catalyzing the synthesis of monounsaturated fatty acids, mainly oleate (C18:1) and palmitoleate (C16:1), by the O_2-dependent desaturation of palmitate (16:0) and stearate (18:0) (Gomez *et al*., 2003). Our group has found that the administration of *S. mexicana* seed oil to spontaneously hypertensive rats reduces body weight, adiposity, and improves blood pressure, adiponectin and triglyceride levels (Herrera-Meza *et al*., 2013).

Given the potential effects that the consumption of sterculic acid might have on lipid metabolism and the fact that there is no information about the fatty acid composition of Mexican Sterculiaceae, the purpose of this study was to determine the fatty acid profile and the physicochemical characteristics of

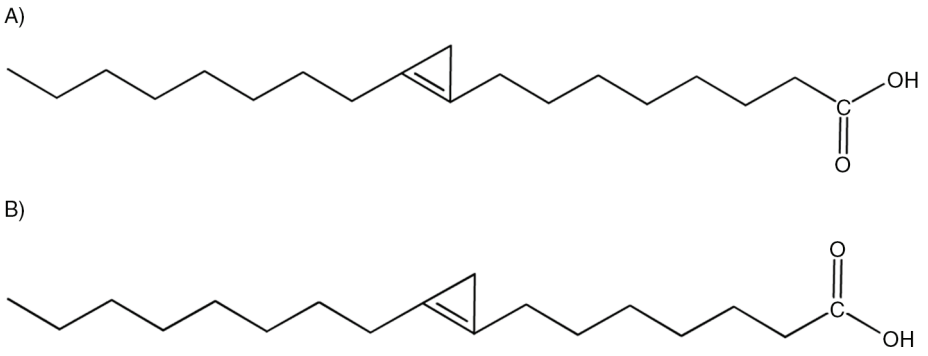

FIGURE 1. Chemical structures of: (A) sterculic acid (C19:1, [8-(2-octyl-1-cyclopropenyl) octanoic acid]) and (B) malvalic acid (C18:1, [7-(2-octyl-1-yclopropenyl) heptanoic acid]).

S. apetala seed oil and to evaluate the effect of roasting on both aspects.

2. MATERIALS AND METHODS

2.1. Sample collection

Seeds from *S. apetala* were collected from two trees in Tolome (19° 16′ 0″ N, 96° 23′ 41″ W, altitude 40 m) in June and July. Results on fatty acid composition were compared to those previously found for *S. mexicana* (Herrera-Meza *et al*., 2013). Seeds from *S. mexicana* were collected from six trees found in Las Choapas (17° 15′ N, 99° 15′ W, altitude 115 m) in February and March. Both localities are located in the state of Veracruz, México. The seeds were stored at 4°C until further processing.

2.2. Seed roasting procedure

In order to analyze the effect of the roasting process, 1000 grams of *Sterculia* seed oil, *S. apetala* seeds were subjected to roasting in a pan exposed to stove heat, with a temperature of 140–150 °C for 20 min with occasional stirring, in a similar way as local people roast the seeds for their own consumption.

2.3. Oil extraction

Natural and roasted seeds were peeled manually and crushed in a mortar and their pulps were mixed with n-hexane for one week, at room temperature. The seeds to solvent ratio was 1:3 (m/v). The solvent was removed using a rotating evaporator at 50 °C.

2.4. Fatty acid analysis

The fatty acids were derived according to Christie (Christie, 1982), using sodium methoxide and methyl acetate. Fatty acid methyl esters (FAME) were analyzed by gas chromatography (Hewlett Packard Model 61800B, GCD system with a capillary column polyethylene glycol, MW 20,000 (Carbo-Wax 20 M), 30 m×0.25 mm×0.25 μm, and mass spectrometry (Agilent Technologies 5975 inert XL model). The initial temperature was 150 °C for 5 min, increased to 210 °C at a rate of 30 °C·min^{-1}, then increased to 213 °C at a rate of 1 °C·min^{-1}, finally increased to 225 °C at a rate of 20 °C·min^{-1} and held for 40 min. Helium was used as a carrier gas, and mass spectra were obtained by electron-impact ionization at 70 eV. The identification of the peaks of each fatty acid was made by comparing the spectra with the GC library (HP-Chemstation NIST 05 Mass Spectral Research Program Version 2.0d) and with fatty acid standards (F.A.M.E mix, C8:C22, 18920-1AMP, Sigma-Aldrich) analyzed under the same conditions. The samples were analyzed in triplicate.

2.5. Selected physicochemical properties

Analyses of specific gravity, iodine value, acid value and saponification value of *S. apetala* natural and roasted seed oil were carried out using the standard methods (AOAC, 1990). The samples were analyzed in triplicate. Refractive index was determined on Abbe, Leica Mark II refractometer, model 10480.

2.6. Statistical analysis

A separate, independent t-test was conducted for the comparison of fatty acid profiles between *S. mexicana* and *S. apetala*, a one sample *t*-test was used to compare these two species and t-test dependent samples (natural and roasted seed oil). We fitted the data into the software Statistica 7.0 (StatSoft, Inc. 1984–2004).

3. RESULTS AND DISCUSSION

The fatty acid composition of *Sterculia apetala* natural seed oil is shown in Table 1. The total ion current chromatogram of the FAMEs from *Sterculia apetala* (A) and *Sterculia mexicana* (B) is shown in Figure 2, and the respective mass spectra in Figure 3. The fatty acid profile was similar to those reported for *S. foetida* (Corl *et al*., 2001) and *S. mexicana* (Herrera-Meza *et al*., 2013) seed oils, so their fatty acid composition is included in tables for comparison purposes. As can be seen in Table 1, the major component in *S. apetala* and *S. mexicana* seed oil was sterculic acid (19:1cyclo), the highest content was found in *S. apetala* (56.3±0.91 vs 51.3±0.29 in *S. mexicana*, $p<0.02$; [9% higher]). The other CPFA, malvalic acid (18:1cyclo), was present in a minor proportion (1.1±0.05 in *S. mexicana* and 1.3±0.06 in *S. apetala*). With respect to other fatty acids, the most abundant was palmitic acid (16:0) followed by oleic acid (18:1) and stearic acid (18:0). Minor but significant differences were found in oleic acid (C18:1) (8.8±0.09 in *S. mexicana* vs 9.5±0.16 in *S. apetala*, $p<0.04$) and linoleic acid (C18:2) (3.8±0.06 in *S. mexicana* vs 2.9±0.11 in *S. apetala*, $p<0.01$). Palmitic acid (C16:0) and stearic acid (18:0) percentages were found similar in both species (Table 1). In comparison to other *Sterculia* species, *S. apetala* and *S. mexicana* showed a high content of sterculic acid (56.3% and 51.3% vs 11.3% in *S. tormentosa*, 30.2% in *S. tragacantha*, 5.3 in *S. striata*, 4% in *S. alata*, 5. 8% in *S. guttata*, 3.2% in *S. villosa*), while a relatively low content was found for malvalic acid (1.3% and 1.1% vs 5.8% in *S. tormentosa*, 5.1% in *S. tragacantha*, 2.3 in *S. striata*, 17.6% in *S. alata*, 2.1% in *S. guttata*, 2.5% in *S. villosa*) (Miralles *et al*., 1993; Nitao *et al*., 2008; Badami *et al*., 1980). Conversely, the fatty acid profiles were more like those found in *S. foetida* seed oil

TABLE 1. Fatty acid profiles of *Sterculia mexicana* and *Sterculia apetala* seed oils with the value of t-test for the independent sample

Fatty acid	*S. mexicana**	*S. apetala*	*t-test*
16:0	21.8±0.18	19.1±4.07	t=0.003, P=0.9
18:0	8.5±0.13	6.1±1.99	t=0.7, P=0.5
18:1	8.8±0.09	9.5±0.16	**t=−4, P=0.04**
18:1cyclo[a]	1.1±0.05	1.3±0.06	t=−1, P=0.40
18:2	3.8±0.06	2.9±0.11	**t=9, P<0.01**
19:1cyclo[a]	51.3±0.29	56.3±0.91	**t=−6, P<0.02**
Other	4.4±0.26	4.8±0.27	t=−1, P=0.33

[a]Presence of cyclopropene ring. C18:1, malvalic acid [7-(2-octyl-1-ylcyclopropenyl) heptanoic acid]; C19:1, sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid]. Values are expressed as mean ± SE (percentage of total fatty acids). *Reported by Herrera-Meza *et al*., 2013.

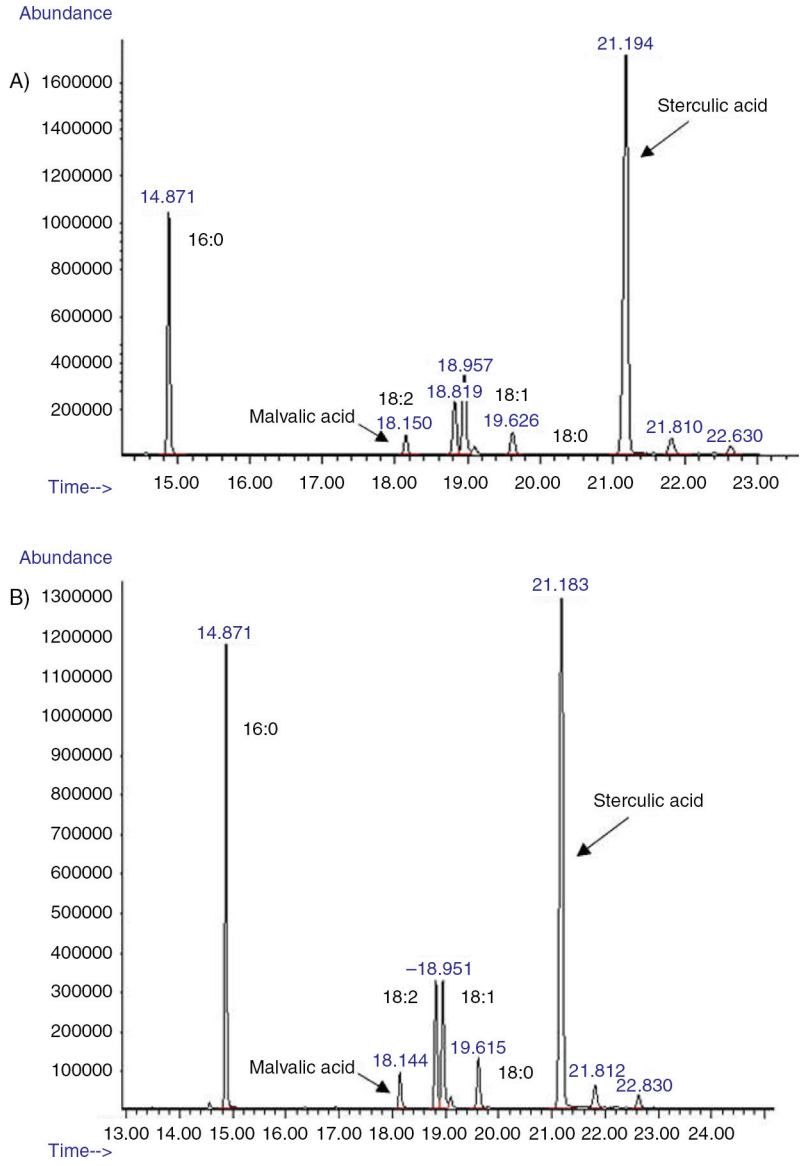

FIGURE 2. Total ion current gas chromatograms of the fatty acids profile of *Sterculia apetala* (A) and *Sterculia mexicana* (B).

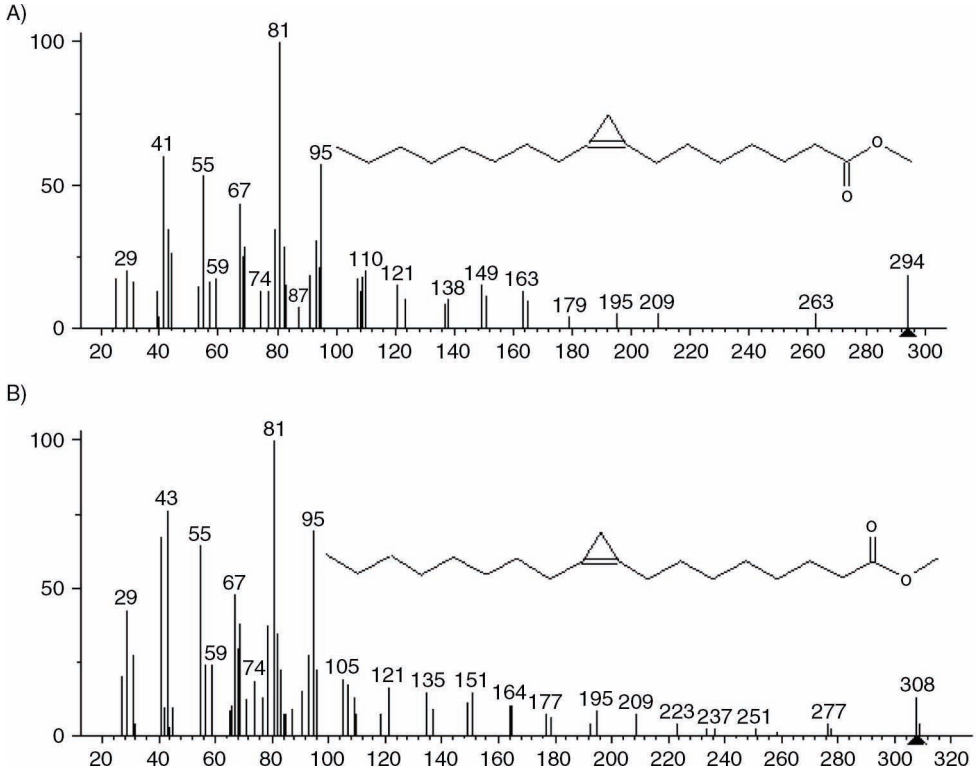

FIGURE 3. Mass spectra of sterculic acid from *Sterculia apetala* (A) and *Sterculia mexicana* (B) seed oil.

(Corl *et al*., 2001) (Table 2). With respect to CPFA, sterculic acid was found in similar proportions, although *S. mexicana* showed a significantly lower amount (56.3±0.91 in *S. apetala*, 51.3±0.29 in *S. mexicana* vs 55.8 in *S. foetida*, p<0.03). *S. mexicana* and *S. apetala* showed a significantly lower amount of malvalic acid (18:1cyclo) (1.1±0.05 in *S. mexicana* [81% lower], 1.3±0.06 in *S. apetala* [80% lower]

TABLE 2. Fatty acid profiles of *Sterculia foetida* seeds oils with the value of t-test for the simple sample

Fatty acid	*S. foetida*[b]	*S. mexicana* t-test	*S. apetala* t-test
16:0	22.9	t=−7, P=0.08	t=−0.3, P=0.7
18:0	1.8	**t=71, P<0.01**	**t=4, P=0.01**
18:1	5.0	**t=59, P<0.01**	**t=39, P<0.01**
18:1cyclo[a]	6.3	**t=−146, P<0.004**	**t=−126, P<0.005**
18:2	5.0	**t=−31, P=0.02**	**t=−26, P=0.02**
19:1cyclo[a]	55.8	**t=−21, P=0.03**	t=−0.4, P=0.7
Other	2.8	t=7, P=0.08	t=9, P=0.06

[a]Presence of cyclopropene ring. C18:1, malvalic acid [7-(2-octyl-1-yclopropenyl) heptanoic acid]; C19:1, sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid]. Values are expressed as percentage of total fatty acids.
[b]Mean values of *S. foetida* reported by Corl *et al*., 2001.

vs 6. 3 in *S. foetida*, p<0.004). A higher content of stearic acid (18:0) (6.1±1.99 in *S. apetala*, 8.5±0.13 in *S. mexicana* vs 1.8 in *S. foetida*, p<0.01) and oleic acid (C18:1) (9.5±0.16 in *S. apetala*, 8.8±0.09 in *S. mexicana* vs 5.0 in *S. foetida*, p<0.01) were found in both *Sterculia* species, while the concentration of linoleic acid was lower than in *S. foetida* (C18:2) (2.9±0.11 in *S. apetala*, 3.8±0. 06 in *S. mexicana* vs 5.0 in *S. foetida*, p<0.02) (Table 2).

The consumption of roasted *Sterculia apetala* seeds among the southeastern Mexican population is very common. Roasting is executed by a handmade process intended to develop a desirable flavor and aroma similar to peanut butter or nuts. We followed the procedure used by local people in order to analyze the effect of the roasting process on *Sterculia apetala* seed oil's fatty acid composition. It has been reported that heat destroys the inhibitory activity of sterculic acid over SCD (Gomez *et al*., 2003), and that vegetable edible oils containing CPFA need to be treated with high temperatures before consumption in order to avoid possible health problems (Bao *et al*., 2003). Results showed that roasting exerted important changes in the seed oil's fatty acid composition. Although sterculic acid was still the main component of the seed oil, its amount diminished by 36% with respect to the natural seed oil (35.9±0.12 in roasted seed oil vs 56.3±0.91 in natural seed oil,

TABLE 3. Fatty acid profiles of natural and roasted seed oil of *Sterculia apetala* with the value of t-test for the dependent sample

Fatty acid	Natural seeds oil	Roasted seeds oil	*t-test*
16:0	19.1±4.07	29.2±0.16	t=59.9, P<0.0002
18:0	6.1±1.99	3.0±0.05	t=54.4, P<0.0003
18:1	9.5±0.16	18.1±0.16	t=53.0, P<0.0003
18:1cyclo[a]	1.3±0.06	2.6±0.016	t=75.4, P<0.0001
18:2	2.9±0.11	5.2±0.03	t=70.8, P<0.0001
19:1cyclo[a]	56.3±0.91	35.9±0.12	t=167.0, P<0.00001
Other	4.8±0.27	5.9±0.024	t=48.9, P<0.0004

[a]Presence of cyclopropene ring. C18:1, malvalic acid [7-(2-octyl-1-cyclopropenyl) heptanoic acid]; C19:1, sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid]. Values are expressed as mean ± SE of percentage of total fatty acids.

p<0.001). The content of malvalic acid was found to have increased by 96% (2.55±0. 016 in roasted seed oil vs 1.2±0. 06 in natural seed oil, p<0.001). We have found that the administration of natural seed oil from *S. mexicana* to spontaneously hypertensive rats provokes an inhibition of SCD activity (Herrera-Meza *et al*., 2013). Whether these modifications may cause a loss in the inhibitory activity of sterculic oil over SCD remains to be investigated. With the exception of stearic acid, the percentage of all the other fatty acids was increased after the roasting procedure (Table 3).

The physicochemical properties of *S. apetala* natural and roasted seed oil are shown in Table 4. The refractive index value of natural seed oil was similar to that reported for common oils such as soybean (1.4) (Bello *et al*., 2011), and was not modified by the roasting procedure. Specific gravity in the natural seed oil was found similar to other edible oils (Bello and Agg, 2012), and was increased after seed roasting. The iodine value is a measurement of the degree of instauration and reflects the susceptibility of oil to oxidation (Bello *et al*., 2011). The oil presented a low iodine value (45.7) which is in accordance with seed oil's fatty acid profile, mainly composed of saturated and monounsaturated fatty acids; it was found to have decreased after roasting (33.9). Since acid value is indicative of the content of free fatty acids in the oil, it is an important variable when considering the quality of oil: the lower the free fatty acids, the better the quality (Ibeto *et al*., 2012). According to the results, there are free fatty acids and triglycerides, methyl esters were obtained from both. *S. apetala* seed oil showed a very high acid value, both in natural (79.4 mg KOH·g^{-1}) and in toasted (87.8 mg KOH·g^{-1}) seed oil; however it could be minimized during refining of the oil. The saponification value is expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify 1 g of sample (Nayak and Patel, 2010). The saponification values of natural and roasted seed oil were 140.4 and 159.3 which indicates that the oil contains high molecular weight fatty acids on average. In consequence, this oil is not suitable for soap manufacturing.

TABLE 4. Physicochemical parameters of *Sterculia apetala* natural and roasted seed oil

Values	Natural oil	Toasted oil
Color	Greenish yellow	Red
Refractive index	1.46±0.0007	1.46±0.0008
Specific gravity	0.9±0.004	1.4±0.07
Iodine value ($gI_2 \cdot 10\ g^{-1}$)	45.7±1.27	33.9±1.03
Acid value (mg KOH·g^{-1})	79.4±0.86	87.8±3.34
Saponification value (mg·g^{-1})	140.4±12.75	159.3±8.49

Values are expressed as mean ± SE.

In conclusion, the cyclopropenoic fatty acids, malvalic and sterculic acids were identified in *Sterculia apetala* seed oil in high amounts, similar to those found in *Sterculia mexicana* and *Sterculia foetida*. The roasting process modified the fatty acid composition of the seed oil; in particular a decrease in the content of sterculic acid was observed. It is noteworthy that *S. mexicana* and *S. apetala* seeds are commonly used in some tropical areas in Mexico for human and animal nutrition, so epidemiological studies are needed to determine possible health effects on the population. To our knowledge, this is the first report of the fatty acid composition of *S. apetala* seed oil.

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