Quantitative analysis of fatty acids in *Prosopis laevigata* flour

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SUMMARY: Ripe mesquite pods are widely consumed by humans and animals in arid and semi-arid areas for their protein, carbohydrate, crude fiber and fat contents. The goal of this work is to identify and to quantify the fatty acid profile of flour from mesquite pods. Structural assignments were confirmed by the analysis of fragmentation patterns of mass spectra obtained by GC-MS. The results showed that 75% of the fatty acids were unsaturated, of which linoleic acid was predominant, while palmitic and stearic acids, and saturated fatty acids were found in minor proportions.

KEYWORDS: *Fatty acid; Linoleic acid; Mesquite;* Prosopis

RESUMEN: *Análisis cuantitativo de ácidos grasos en harina de* **Prosopis laevigata**. Las vainas de mezquite maduro son ampliamente consumidas por humanos y animales en las zonas áridas y semiáridas por su contenido de proteínas, carbohidratos, fibra cruda y grasas. El propósito de este trabajo es identificar y cuantificar el perfil de ácidos grasos de harinas de vainas mezquite. La estructura química fue confirmada mediante el análisis de los fragmentos del espectro de masas obtenidos por GC-MS. Los resultados mostraron que el 75% de los ácidos grasos fueron insaturados, de los cuales, el ácido linoleico predomina mientras que el ácido pálmico y esteárico, ambos ácidos grasos saturados, fueron encontrados en menor proporción.

PALABRAS CLAVE: *Ácido linoleico; Ácidos grasos; Mesquite;* Prosopis

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1. INTRODUCTION

Mesquite (*Prosopis laevigata* H. & B.) is a species of the genus *Prosopis*, and is widely distributed throughout arid and semi-arid areas worldwide. In Mexico, it is spread across the Central High Plateaus in the north, the lower reaches of Tamaulipas and in parts of Oaxaca, Morelos, Puebla and Chiapas (Pérez *et al*., 2013).

This variety has the ability to form organic matter and fix nitrogen, thus benefiting the agroforestry ecosystem (Corona *et al*., 2000). Mesquite is useful as a fuel wood, and ripe pods are avidly consumed by all ruminant species. The pods have been a historic source of food for human populations; traditionally, flour and dough are made with the dried or toasted pulp from ripe pods (Alves *et al*., 2016). The pods contain 7–22% protein, 30–75% carbohydrate, 11–35% crude fiber, 1–6% fat and 3–6% ash (Anttila *et al*., 1993; Galera *et al*., 1992; Oduol *et al*., 1986); although the polyunsaturated fatty acid (PUFA) content is unknown.

PUFAs perform multiple physiological functions in cell membranes and are significantly involved in regulating important membrane properties. PUFAs serve as precursors to fatty acids known to be important mediators in immune systems and pathological and inflammatory processes. Some PUFA, like linoleic and linolenic acid, must be consumed in the diet since they cannot be synthesized by humans (Corona *et al*., 2000; Simopoulos, 2002).

Few works on *Prosopis* are available in the literature, most of them focused on nutritional characterization. The effect of the drying temperature of the pods on the protein content, amino acids and sensory properties have been evaluated in some works. In fresh seeds the content of free sugars, crude protein and fatty acids has been reported. In other varieties of *Prosopis*, antioxidant capacity, genotoxicity and polyphenol content have been reported in *Prosopis nigra* flour (Gallegos-Infante *et al*., 2013; Cardozo *et al*., 2010).

Hence, the aim of this work is to identify and to quantify the fatty acids in mesquite flour.

2. MATERIALS AND METHODS

2.1. Materials

Ripe mesquite pods (*P. laevigata* H. & B.) were harvested in Oaxaca, Mexico (96°52' WL, 17°15' NL) from April–May. The moisture content was determined according to the AOAC method (2000) and was expressed as g water /g dry solid (g_w/g_{ds}).

The pods were dried at 50±0.22 °C and 70±0.68 °C at an air flow rate of 2 m/s in a convective dryer (Mexican patent 304462) for 7 and 5 h, respectively. All parts of the mesquite pod (exocarp, mesocarp and seed) were ground using a mill for legumes (HC-2000Y) to crush the dried mesquite pods for 20 seconds in order to reduce the heating time and prevent oxidation. To obtain the flour a sieve mesh 60 (0.250 mm) A.S.T.M. was used.

Lipid extraction. The Soxhlet method was used to determine the total fat content (A.O.A.C., 1990). Briefly, 10g of flour were placed in Whatman cellulose extraction thimbles. The thimbles were loaded into the main chamber of the Soxhlet extractor, and 80 mL of petroleum ether (Sigma Aldrich, St Louis, MO, 69 USA) were placed in a distillation flask and heated at 48 °C for 8 h. Total lipids were expressed as g of lipids/100 g of mesquite flour.

2.2. Fatty acid methyl esters (FAMEs)

Transesterification. The transesterification procedure was carried out according to (Martinez *et al*., 2003). A volume of 800 μL of $CHCl_3$-MeOH (2:1 v/v) was added to 0.1 g of lipid sample. HCl (37%, w/w; 0.33 mL) was diluted with 4.6 mL of methanol to make 5 mL of 8.0% (w/v) HCl. Then, 1 mL of the 8% HCl reagent was added to an aliquot of 200 μL of the previous solution, and it was heated at 80 °C for 20 min. The solution was left to reach room temperature, then 200 μL of distilled water and 2 mL of hexane were added. The organic phase was separated, dried with 0.5 g of anhydrous magnesium sulfate, evaporated and resuspended in 2 mL of hexane.

Gas chromatographic analysis. The FAMEs of total lipids were analyzed on a Perkin Elmer Clarus 580 (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector (FID), using a fused silica capillary column (SP™-2380, 30 m i.d. × 0.25 mm f.d. with a 0.20 μm film thickness) from Supelco (Bellefonte, PA, USA). The column oven temperature was programmed to 60 °C, 2.0 min; 60-185 °C, 4.0 °C/min; 185 °C, 16.0 min. Injector temperature was 220 °C. The carrier gas was helium at a flow rate of 1.2 mL/min, and the injector split ratio was 100:1. Detector temperature was set at 220 °C.

The separated FAMEs were identified by comparing their retention times (t_R) with those of the standard FAME Mix (Supelco Inc., Bellefonte, PA, USA). Quantitative analysis of the fatty acids was performed using heptadecanoic acid methyl ester as an internal standard.

Determination of iodine values. Iodine values were calculated from the fatty acid composition (Hashim *et al*., 1993) using the formula: I.V. = (% oleic × 0.8601) + (% linoleic × 1.7321) + (% eicosenoic × 0.7854).

Fatty acid quantification. The AACC 58-19 (1999) method was used for quantification of fatty acids. A response factor R_i was determined using equation 1:

$$R_i = Ws_{C_{18}H_{36}O_2} \times \frac{P_{Si}}{Ps_{C_{18}H_{36}O_2}} \qquad (1)$$

where R_i is the response factor for each fatty acid i (mg/mL); Ps_i is the peak area of the individual fatty acid i $(\%)$; $Ps_{C_{18}H_{36}O_2}$ is the peak area of the $C_{18}H_{36}O_2$ internal standard (%); $Ws_{C_{18}H_{36}O_2}$ is the amount of internal standard $C_{18}H_{36}O_2$ in the solution (mg/mL).

The concentration of each fatty acid as methyl ester equivalents in the esterified total fat sample was calculated using equation 2:

$$C_{FAME} = R_i \times \frac{Ve}{W_{etf}} \qquad (2)$$

where C_{FAME} is the concentration of fatty acids as methyl ester equivalents (mg FAME/mg of esterified total fat sample); R_i is the response factor for each fatty acid i (mg/mL); Ve is the volume of extraction solvent (mL); W_{etf} is esterified total fat (mg).

The concentration of each fatty acid as methyl ester equivalents (FAMEs) in mesquite flour was calculated using equation 3:

$$C_{FAME\ FLOUR} = \left[\frac{C_{FAME} \times W_{etf} \times 100}{1000} \right] \qquad (3)$$

Where: $C_{FAME\ FLOUR}$ is the concentration of fatty acid as methyl ester equivalents in mesquite flour (g FAME/100 g of flour); C_{FAME} is the concentration of fatty acid as methyl ester equivalents (g FAME/g of esterified total fat sample); W_{etf} is the weight of esterified total fat (g).

2.3. GC-MS analysis

GC-MS analysis was performed using a Perkin Elmer Clarus 580 coupled to a Clarus SQ 8S selective mass detector (Shelton, CT, USA) using the same temperature program as described in section 2.3. The column outlet was directly connected to the ion source of the mass spectrometer operating at 200 °C. Source fragmentation was done by electron ionization (EI) using an ionization energy of 70 eV, with a scan range of 50–450 amu (atomic mass units) and a scan rate of 1.80 scans per second. Data was visualized using Turbo Mass Version 6.1.0 software.

2.4. Experimental design and data analysis

One-way design was used to evaluate the effect of drying temperature on the concentration of fatty acids. Significant difference was calculated using ANOVA conducted at a level of $p < 0.05$ and the software NCSS11 Data Analysis (USA). The Duncan test was conducted to evaluate differences among individual means. Values are provided as mean of 3 replicates.

3. RESULTS AND DISCUSSION

3.1. Moisture content of pods and flour

The initial moisture content of the raw pods was 0.2234 ± 0.02 g_w/g_{ds}, the flour dried at 50 °C reached 0.15 ± 0.01 g_w/g_{ds} and flour dried at 70 °C reached 0.1 ± 0.0 g_w/g_{ds}. The drying kinetics (Figure 1) showed that the drying time was longer for a drying temperature of 50 ± 0.22 °C.
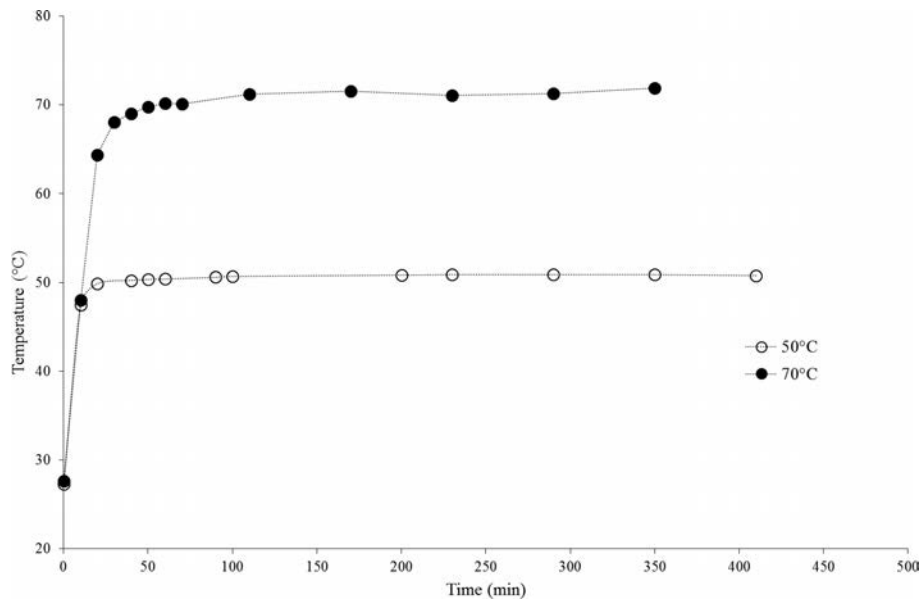

FIGURE 1. Drying kinetics of mesquite pods.

3.2. GC-FID analysis

The total fat content was 2.64±0.23 and 1.87±0.56 g/100 g of mesquite flour at 50±0.22 °C and 70±0.68 °C. These results are higher than those reported for wheat flour (1.6 $g_{total\,fat}$/100 g_{ds}) and corn flour (1 $g_{total\,fat}$/100 g_{ds}) (Muñoz, 2014).

The fatty acid composition of mesquite flour is presented in Figure 2. The temperature had a significant effect (α=0.05) on the concentration of fatty acids (Table 1). 74.4% of unsaturated fatty acids were found in samples dried at 50±0.22 °C, of which linoleic acid was predominant; while at 70±0.68 °C, 75.9% were unsaturated fatty acids. In terms of the relative percentage, SFA and PUFA decreased in mesquite flour obtained from pods dried at 70±0.68 °C; while the concentration of FSA, MUFA and PUFA in total flour fat, significantly decreased (α=0.05) as the drying temperature increased, confirming the thermal degradation. PUFAs were more affected than MUFAs; although oleic acid was the most thermally stable fatty acid with a loss of 10%. The instability of polyunsaturated fatty acids at these temperatures leads to chemical transformations, such as oxidation.

Marangoni *et al.*, (1986) reported similar results for *Prosopis juliflora* (DC) seeds and pods, which contain a high proportion of unsaturated fatty acids, predominately linoleic acid. *P. juliflora* is a tree of the Fabaceae family, as is *P. laevigata*. From a nutritional perspective, mesquite flour contains essential dietary fatty acids (C18:1; C18:2, C18:3) with important health benefits (Matsumoto *et al.*, 2017; Simopoulos, 2002). In addition, the concentration of essential fatty acids is higher than those reported for corn and wheat flour (Muñoz, 2014).

The saturated fatty acids palmitic (C16:0) and stearic acid (C18:0) were present at 19.7% and 1.4%, in samples dried at 50±0.22 °C. Saturated fatty acids give product stability and resistance to rancidity and oxidation (Belén *et al.*, 2001).

Table 1 shows the FAME for mesquite flour dried at 50±0.22 °C and 70±0.68 °C. The thermal treatment of mesquite flour had an effect on fatty acid degradation. The higher drying temperature (70±0.68 °C) caused a concentration loss in FAMEs after 7 h of drying; moreover, only oleic acid showed little variation at both drying temperatures. According to Fournier *et al.*, (2006) UFAs are unstable, and thermal treatment induces chemical transformations like oxidation; however, the rate and ability to perform these chemical reactions are determined by

TABLE 1. The effect of temperature on fatty acid (FAME) concentration in flour

FAME	Concentration in total fat		Concentration relative	
	50 °C	70 °C	50 °C	70 °C
	g/g total fat		% relative	
Palmitic C16:0	0.51±0.01[a]	0.35±0.00[b]	19.7	18.8
Palmitoleic C16:1	0.03±0.00[a]	0.01±0.00[b]	1.4	1.0
Stearic C18:0	0.12±0.00[a]	0.08±0.00[b]	4.6	4.3
Oleic C18:1	0.49±0.05[a]	0.44±0.00[b]	18.9	23.6
Linoleic C18:2	1.21±0.03[a]	0.81±0.00[b]	46.2	43.6
Linolenic C18:3	0.24±0.00[a]	0.16±0.00[b]	9.3	8.7
TOTAL SFA	0.66±0.00	0.44±0.00	25.7	24.1
TOTAL MUFA	0.49±0.01	0.44±0.00	18.9	23.6
TOTAL PUFA	1.45±0.01	0.97±0.00	55.5	52.3

[a,b] The same letters in different temperature conditions indicate no significant difference. Duncan test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in triplicate
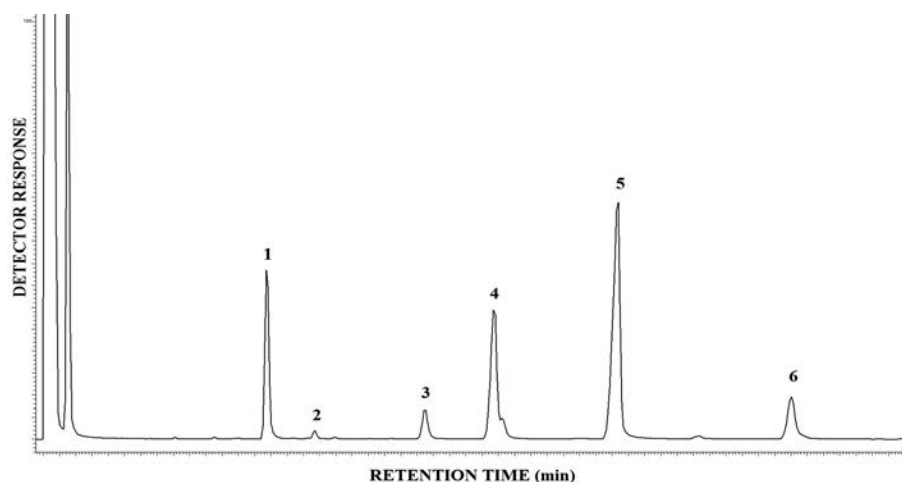

FIGURE 2.   GC chromatogram of fatty acids in mesquite flour: 1: palmitic acid (C16:0), 2: palmitoleic (C16:1), 3: stearic (C18:0), 4: oleic (C18:1), 5: linoleic (C18:2) and 6: linolenic (C18:3).

conjugated (unsaturated) double bond distribution. Linoleic acid (C18:2) is twice as liable to oxidation as oleic acid (C18:1), due to the presence of two active methyl groups in its chemical structure (Baley, 1984).

Fatty acids play multiple functions in the body, influence brain functioning, cardiovascular health, digestion, allergies, immunity, immune system, vision, etc. Of the total energy required for human health, an adequate consumption of essential fatty acids should be 2% linoleic acid and 1% linolenic acid. This corresponds to approximately 0.5 g / day of ω-3 PUFA´s (Rustan *et al.*, 2005). The concentration of PUFAs provided by the mesquite flour sufficiently satisfies the minimum requirements to avoid clinical symptoms of deficiency.

3.3. Iodine values

The iodine index of mesquite flour was 98.1±0.02 and 97.9±0.07 g/100g oil for 50±0.22 °C and 70±0.68 °C, respectively; both results are higher

than those reported by Douglas *et al.*, (2004) for píritu seed (*Bactris piritu*) flour, but closer to the values reported for blackberry, cotton, soybean, sesame and peanut oils. These last have a higher proportion of unsaturated fatty acids, increasing their susceptibility to oxidative processes.

3.4. GC-MS analysis

Structural assignments were based on direct comparison of mass spectral data with profiles from the National Institute of Standards and Technology (NIST MS Search 2.0), and confirmed by the analysis of fragmentation patterns of mass spectra.

Total ion chromatograms of hexane fractions at 50±0.22 °C and 70±0.68 °C show the same six major peaks at retention times (t_R) of 2.37, 2.60, 3.11, 3.45, 4.04 and 4.88 min (Figure 3, Table 2).

The mass spectrum of peaks at t_R 2.37 min showed an ion at *m/z* 74 (base peak) as a result of site-specific rearrangement of atoms, in which the
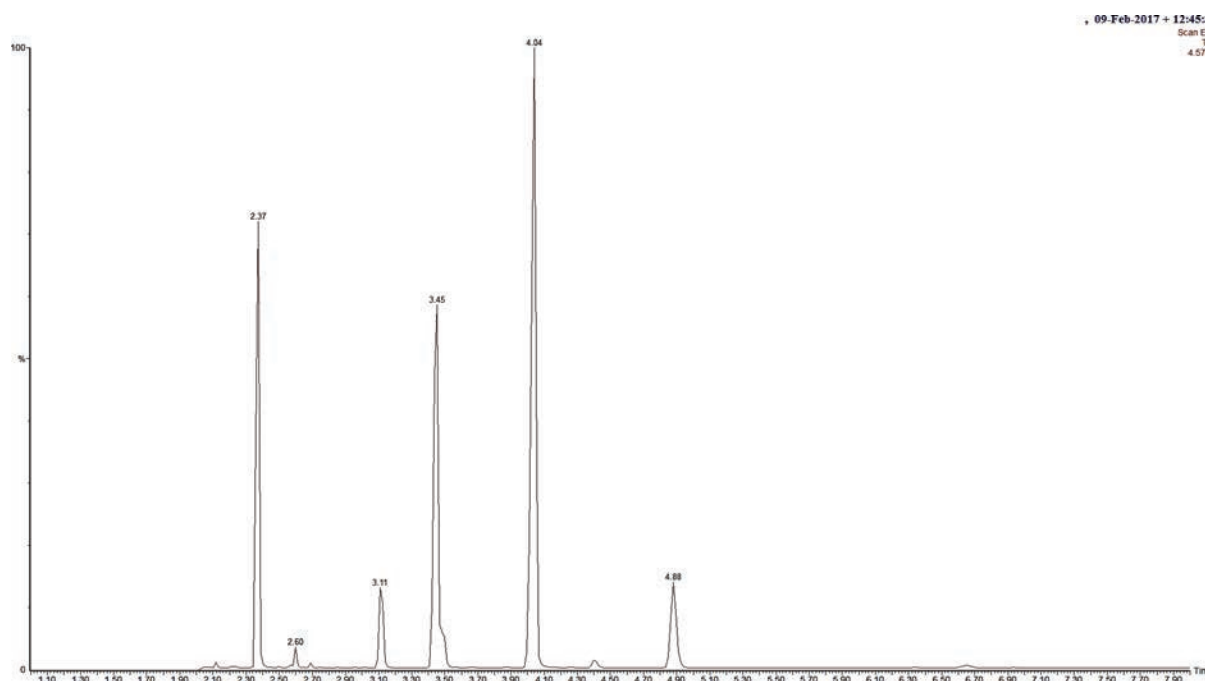

FIGURE 3. Total ion chromatogram (TIC) of hexane fraction of mesquite flour with six major peaks at retention times (t_R) 2.37 min, 2.6 min, 3.11 min, 3.45 min, 4.04 min and 4.88 min.

TABLE 2. Chemical composition of fatty acids from mezquite flour

Compound	t_R	Mol Ion m/z	Fragments Ion m/z
Hexadecanoic acid methyl ester	2.37	270	239,227,213,143,129,87,74
9-Hexadecenoic acid, methyl ester, (z).	2.60	268	236,194,152,74,69,55,41
Octadecanoic acid, methyl ester	3.11	298	255,199,143,87,74,55
9-octadecenoic acid methyl ester	3.45	296	264,222,180,97,69,55
9,12-octadecadienoic acid (z,z)-methyl ester	4.04	294	263,220,150,95,67
9,12,15-octadecatrienoic acid, methyl ester(z,z,z)	4.88	292	261,236,149,135,121,108,95,79,67

γ-hydrogen from the aliphatic chain is transferred to the carbo-methoxy group, through a sterically-favored six-membered transition state (McLafferty rearrangement) followed by Cα–Cβ bond cleavage. The ions at m/z 270, 241, 239, 227 and 74 are characteristic of hexadecanoic acid methyl ester (Figure 4), which has the formula $C_{17}H_{34}O_2$.

The peak at t_R 2.60 min displayed a molecular ion at m/z 268, suggesting a structural formula of $C_{17}H_{32}O_2$. The fraction was determined by diagnostic ion peaks at m/z 236, 194, 152, 74, 69, 55 and 41. The ion at m/z 55 was the base peak. The ions are characteristic of 9-hexadecenoic acid, methyl ester, (z) (Figure 5).
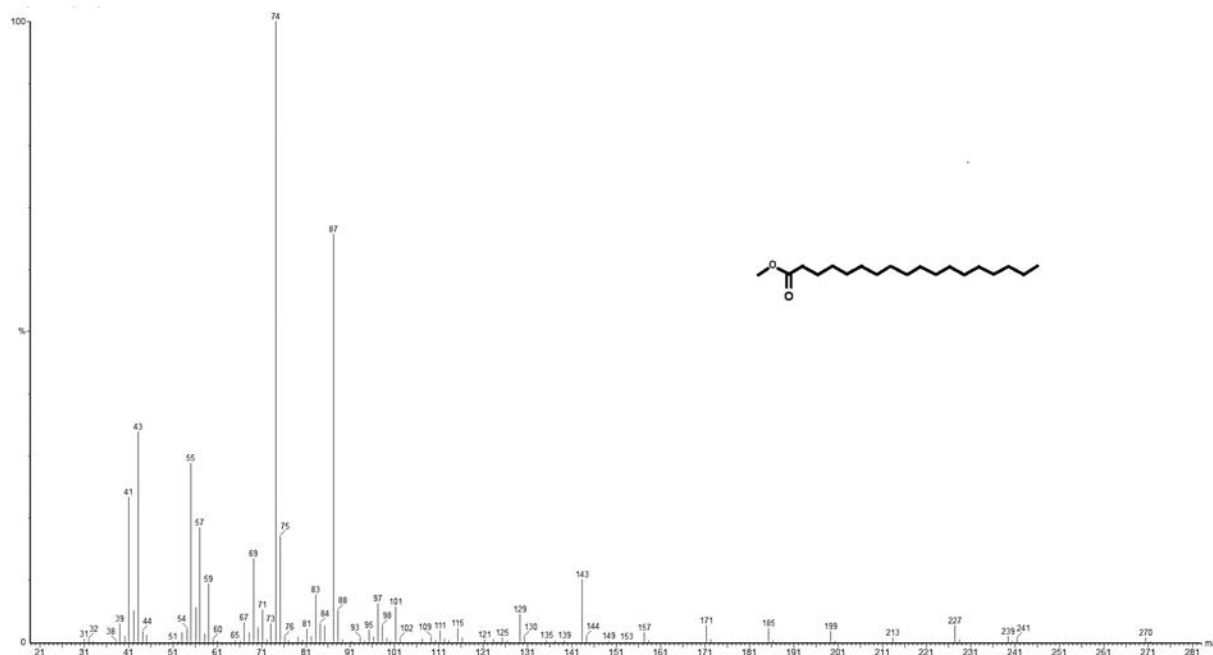

FIGURE 4.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 2.37 min was specific to hexadecanoic acid methyl ester.
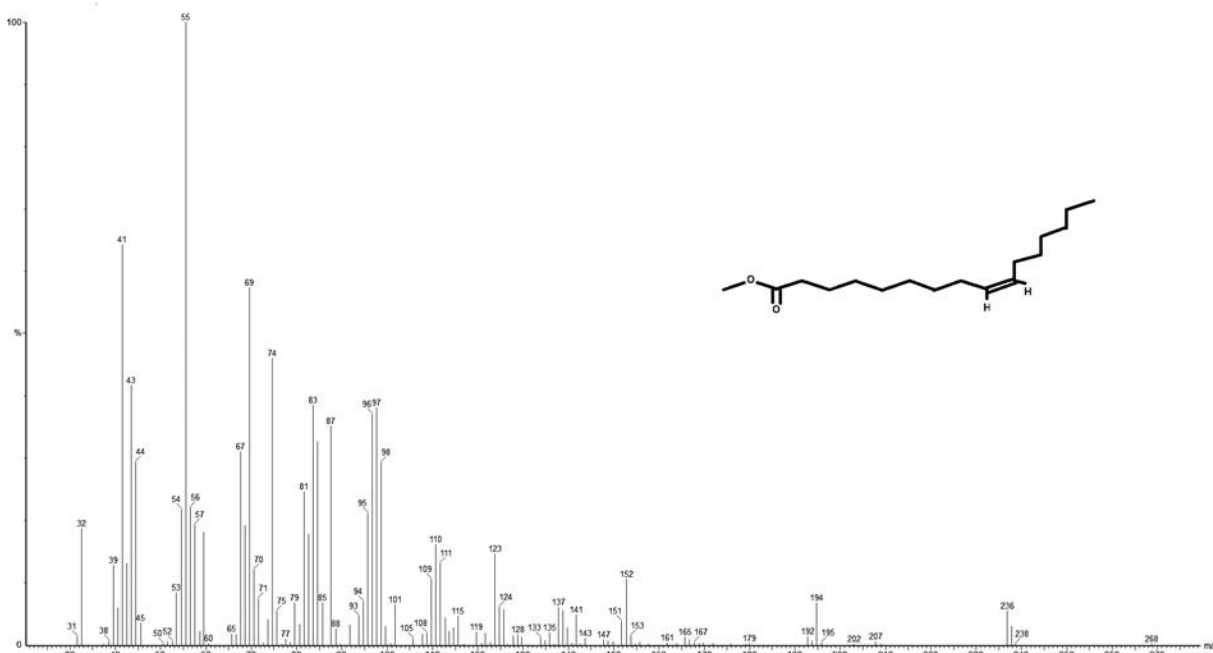

FIGURE 5.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 2.60 min was specific to 9-Hexadecenoic acid, methyl ester, (z).

Analysis of the chromatographic peak at t_R 3.11 min revealed a prominent fragment ion at m/z 74 (base peak fragments characteristic of the mechanism of γ-hydrogen shift), typical of long-chain FAMEs. Other significant fragment ions were observed at m/z 55, 87, 143, 199 and 255, suggesting a structural formula for octadecanoic acid, methyl ester of $C_{19}H_{38}O_2$ (Figure 6).

The peak at t_R 3.45 min displayed a molecular ion at m/z 296, suggesting a structural formula of $C_{19}H_{36}O_2$. The ion at m/z 55 was the base peak. The fragment at m/z 55 indicated a loss of m/z 241(M^+- C_2H_5), the fragment at m/z 69 (M^+- C_3H_7) and other ions at m/z 97, 180, 222 and 264, thus identifying the compound as 9-octadecenoic acid methyl ester (Figure 7).
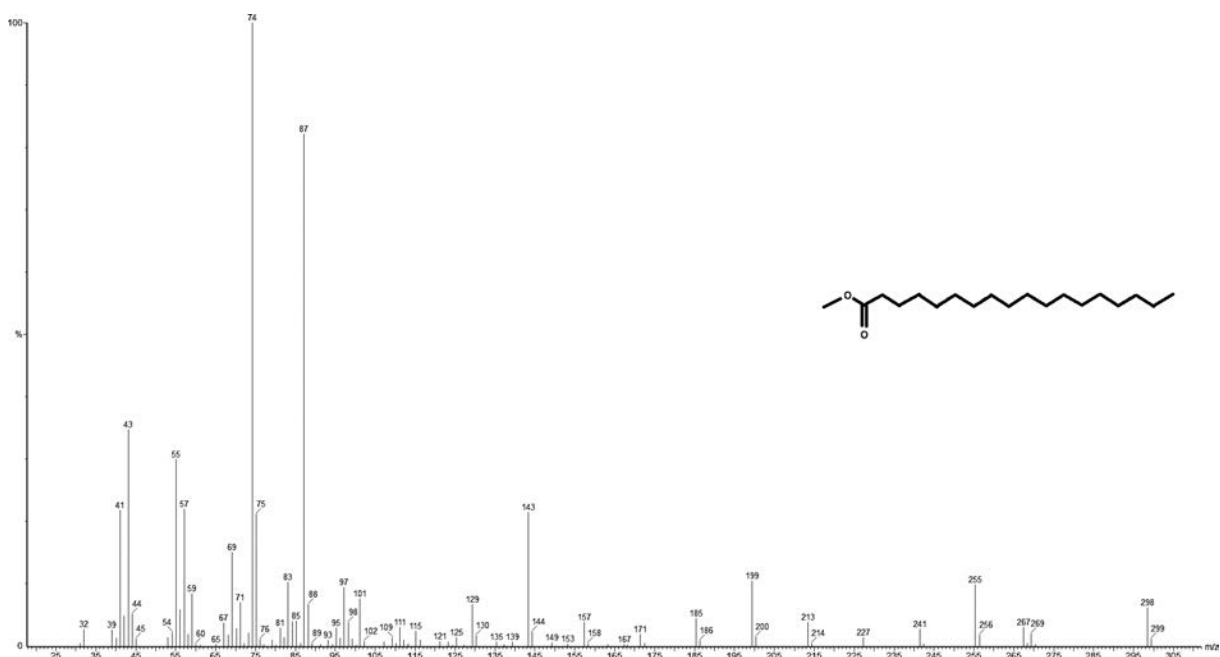

FIGURE 6.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 3.11 min was specific to Octadecanoic acid, methyl ester.
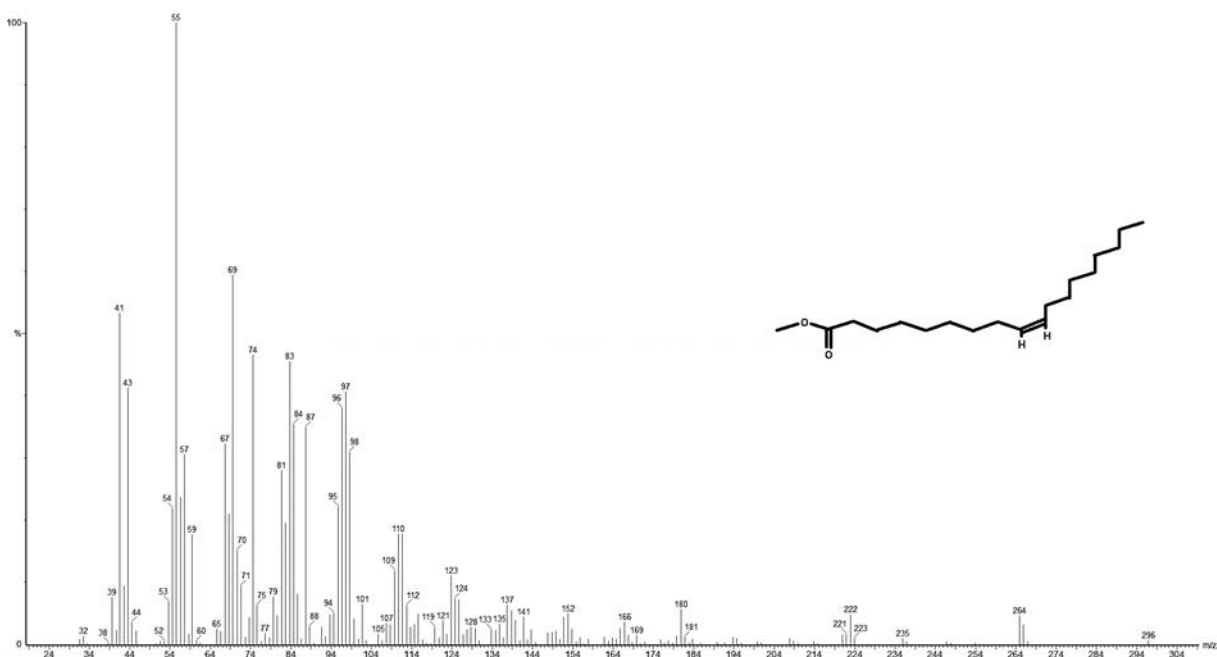

FIGURE 7.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 3.45 min was specific to 9-octadecenoic acid methyl ester.

The mass spectrum of the peak at t_R 4.04 min showed an ion at *m/z* 67 (base peak) (Figure 8). The fragment at *m/z* 67 indicated the loss of a 227 mass $C_{14}H_{27}O_2$ ion; other homologous series of related ions at *m/z* 67, 95, 150, 220 and 263, formed by the loss of neutral aliphatic radicals of the general formula $[(CH_2)n\ COOCH_3)]^+$, which suggested a structural formula for 9,12-octadecadienoic acid (z,z)-methyl ester of $C_{19}H_{34}O_2$.

The identity of the compound represented by the peak at t_R 4.88 min was determined by diagnostic ion peaks at *m/z* 79, 108, 236, 261 and 292. The ion at *m/z* 79 was the base peak. The ion at *m/z* 108 is an omega ion which defines methyl esters of PUFAs with an *n*-3 terminal group. These distinct ions were typical of an *n*-3 homo-allylic unsaturated fatty acid of the molecular formula $C_{19}H_{32}O_2$, called 9(Z)12(Z)15(Z)-octadecatrienoic acid, methyl ester (Figure 9).
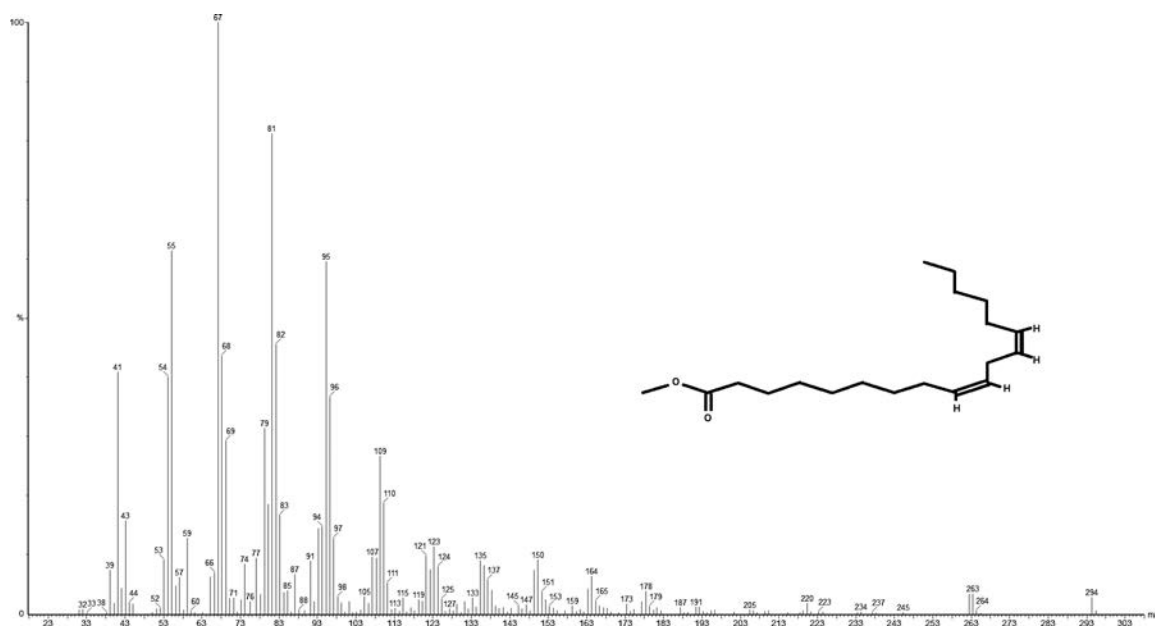

FIGURE 8.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 4.04 min was specific to 9,12-octadecadienoic acid (z,z)-methyl ester.
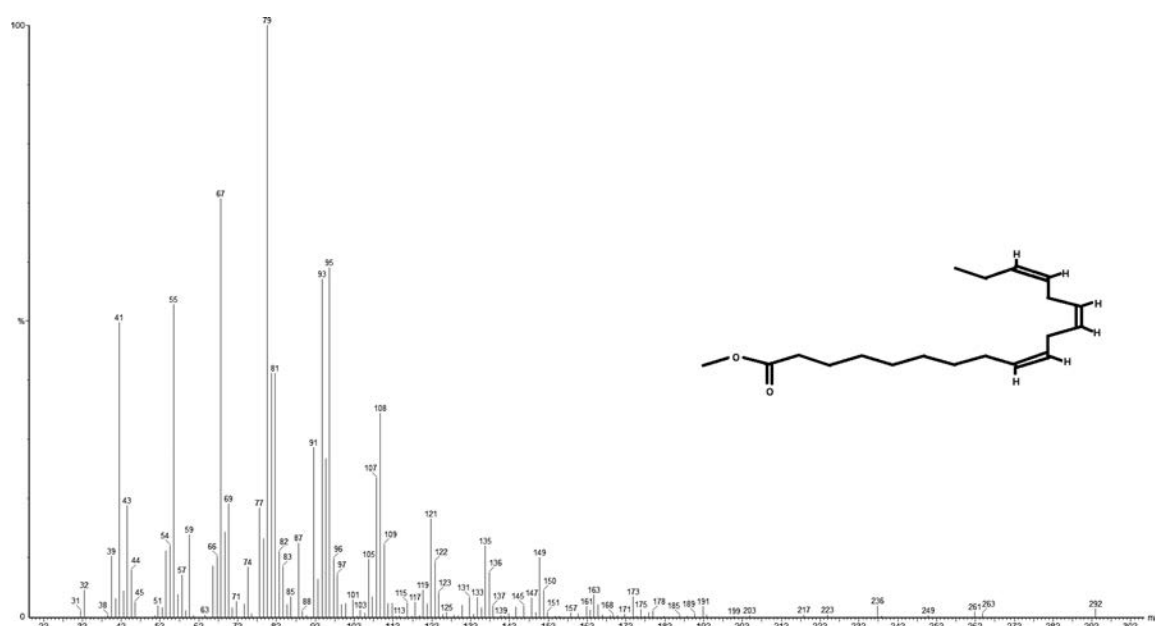

FIGURE 9.   Mass spectrum. Ion fragmentation pattern for spectral peak at t_R 4.88 min was specific to 9,12,15-octadecatrienoic acid, methyl ester(z,z,z).

4. CONCLUSIONS

The fatty acid profile of flours obtained from pods harvested in Oaxaca from April – May were studied in this work. The drying temperature of the pods influenced the concentration of FAME, but did not affect the composition, suggesting that there is no significant decomposition of FAME.

The loss in FAME in the flour obtained from pods dried at 50 °C was reduced by almost half compared to drying at 70 °C. The fatty acids in mesquite flour were predominately unsaturated fatty acids and consisted mainly of linoleic acid. Linoleic acid is an important n-6 fatty acid in the diet, an essential fatty acid that enzymes of the human body cannot synthesize. Mesquite flour is an important source of PUFAs in the diet of consumers in arid zones where the mesquite tree is endemic.

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