JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Five Typical Agave Plants Used To Produce Mezcal through Their Simple Lipid Composition Analysis by Gas Chromatography

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Five agave plants typically used in Mexico for making mezcal in places included in the Denomination of Origin (Mexican federal law that establishes the territory within which mezcal can be produced) of this spirit were analyzed: *Agave salmiana* ssp. *crassispina*, *A. salmiana* var. *salmiana*, *Agave angustifolia*, *Agave cupreata*, and *Agave karwinskii*. Fatty acid and total simple lipid profiles of the mature heads of each plant were determined by means of a modified Bligh−Dyer extraction and gas chromatography. Sixteen fatty acids were identified, from capric to lignoceric, ranging from 0.40 to 459 μg/g of agave. Identified lipids include free fatty acids, β-sitosterol, and groups of mono-, di-, and triacylglycerols, their total concentration ranging from 459 to 992 μg/g of agave. Multivariate analyses performed on the fatty acid profiles showed a close similarity between *A. cupreata* and *A. angustifolia*. This fact can be ascribed to the taxa themselves or differences in growing conditions, an issue that is still to be explored. These results help to characterize the agaves chemically and can serve to relate the composition of mezcals from various states of Mexico with the corresponding raw material.

KEYWORDS: Agave; mezcal; fatty acids; simple lipids

INTRODUCTION

Agave genus has a millenary history in Mexico. These plants have served a wide range of uses along Mexican cultural development, from alimentary purposes to medicinal, textile, construction, and even ornamental ones (*1*−*3*). The genus belongs to the order Asparagales and the family Agavaceae; the total number of species has been subjected to discussion because of the controversy over taxonomic delimitations between genera. García-Mendoza (*6*) has recognized the existence of 200 species plus 47 infraspecific categories, making a total of 247 taxa, 75% of which are found in Mexico, their center of origin (*4*−*6*).

Mezcal is a distilled agave beverage with rising consumption and has been protected by the Denomination of Origin since 1994 to be produced only in certain Mexican states. This protection was established in that year as federal law. Also, in 1997, Mexico agreed with the European Union on the mutual recognition and protection of the Denominations of Origin in the spirits sector. According to the declaration of protection, mezcal can be made from several agave species, so Mexican producers make use of the available kind in their region (*7, 8*).

Agave angustifolia from the state of Oaxaca is probably the most known mezcal agave, given the popularity of its drink; in the same state, *Agave karwinskii* is also employed. *Agave salmiana* is widely used in San Luis Potosí and Zacatecas (and it is the premier source for "pulque", the fermented juice of agave, in central Mexico) and so is *Agave cupreata* in Guerrero. Another commonly used agave is *Agave duranguensis* in Durango. Other species like *Agave potatorum* have been exploited to make the spirit but in smaller proportions and within smaller regions (*1, 9*).

Mezcal production begins with the harvesting of the mature heads of agave (8−12 years), which are also known as "piñas", due to their physical resemblance to a pineapple. Plants are castrated 1−2 years before by removing the tender flower stalk ("quiote") when it starts to grow, for the piñas to fatten and ripen. They are cut into halves or quarters and commonly baked in rock-lined pits in the ground; next, they are crushed to extract the juice, which is then left to ferment and finally distilled. The Mexican Official Norm of mezcal (*10*) considers two types: 100% agave mezcal, which comes from fermenting and then distilling just the agave juice with some water, and "mixto" mezcal, where other types of sugars (up to 20%) have been added to the must. With regard to the scientific research, it is to be noted that there have been only few studies related to mezcal composition (*11, 12*) and even fewer to its raw material, the agave (*13, 14*), probably because of focusing the research on its cousin drink, the tequila, the predominant agave spirit on the market. It differs from mezcal because of its mechanical, more sophisticated production process and the species (only one) that can be used, *A. tequilana* Weber var. azul.

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10.1021/jf802141d CCC: $40.75
Published on Web 02/13/2009

Table 1. Analyzed Samples and Their Origins

agave	locality name	state	latitude, N/longitude, W
A. salmiana Otto ex Salm-Dyck ssp. *crassispina* (Trel.) Gentry	Charcas	San Luis Potosí	23°07′/101°06′
A. salmiana var. *salmiana* Otto ex Salm-Dyck	San Felipe	Guanajuato	21°09′/101°40′
A. angustifolia Haw	Santiago Matatlán	Oaxaca	16°51′/96°22′
A. cupreata Trel & A. Berger	Chilapa	Guerrero	17°36′/99°11′
A. karwinskii Zucc.	Santa Catarina	Oaxaca	16°47′/96°38′

Table 2. Modified Bligh−Dyer Extraction: Volumes of Solvents

agave	water (g/50 g of sample)	CH_2Cl_2 1st addition (mL)	CH_3OH (mL)	CH_2Cl_2 2nd addition (mL)	water (mL)
A. salmiana ssp. *crassispina*	33.44	42	84	42	42
A. angustifolia	42.62	53.5	107	53.5	53.5
A. cupreata	30.92	39	78	39	39
A. karwinskii	33.11	41	82	41	41
A. salmiana var. *salmiana*	36.54	46	92	46	46

In this work, we analyze the simple lipid composition of the mature heads of the five most representative agave plants from which mezcal is made. Chemical characterization can contribute to explain differences between mezcals made by different producers in distinct regions, also favoring their authentication. Simple lipids are important for this aim given their property of generating compounds like fatty acids and FAEEs along the production process of mezcal. The latter is a very important organoleptic group because it presents sweet, floral, and fruity aromas with very low odor thresholds (*15*). This work is also linked with the field of agave chemotaxonomy as a starting point for identification and subsequent classification of the species through the study of their chemical components like fatty acids and total simple lipids, an area that has been neglected until now. We compare the fatty acid profiles of the five plants under study through multivariate analyses like PCA and cluster analysis, finding out the species with major resemblance, which is expected to influence some characteristics (like similarities in FAEEs composition) of the end products.

MATERIALS AND METHODS

Reagents, Solvents, and Standards. Analytical grade dichloromethane, methanol, hexane, butanol, pyridine, hydrochloric acid, potassium hydroxide, sodium sulfate, and sodium chloride were from J. T. Baker (Xalostoc, Edo. de México, Mexico). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and hexamethyldisilazane (HMDS) were from Regis (Morton Grove, IL).

Fatty acid ethyl esters (FAEEs) ethyl caprylate (EE8), ethyl caprate (EE10), ethyl laurate (EE12), ethyl tridecanoate (EE13), ethyl myristate (EE14), ethyl pentadecanoate (EE15), ethyl palmitate (EE16), ethyl palmitoleate (EE16:1), ethyl margarate (EE17), ethyl stearate (EE18), ethyl oleate (EE18:1), ethyl linoleate (EE18:2), ethyl linolenate (EE18:3), ethyl heneicosanoate (EE21), ethyl behenate (EE22), and ethyl lignocerate (EE24) were from NU-CHEK-PREP (Elysian, MN). Fatty acids caprylic (C8), capric (C10), lauric (C12), tridecanoic (C13), myristic (C14), pentadecanoic (C15), palmitic (C16), palmitoleic (C16:1), margaric (C17), stearic (C18), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), heneicosanoic (C21), behenic (C22), and lignoceric (C24) were from NU-CHEK-PREP.

The monoacylglycerols (MG) used were glycerol monocaprylate (MG_8), glycerol monomyristate (MG_{14}), glycerol monopalmitate (MG_{16}), glycerol monostearate (MG_{18}), glycerol monooleate ($MG_{18:1}$), and glycerol monolinoleate ($MG_{18:2}$). The diacylglycerols (DG) used were glycerol dicaprylate (DG_8), glycerol dimyristate (DG_{14}), glycerol dipalmitate (DG_{16}), glycerol distearate (DG_{18}), glycerol dioleate ($DG_{18:1}$), and glycerol dilinoleate ($DG_{18:2}$). The triacylglycerols (TG) used were glycerol tricaprylate (TG_8), glycerol trilaurate (TG_{12}), glycerol trimyristate (TG_{14}), glycerol tripalmitate (TG_{16}), glycerol tristearate (TG_{18}), glycerol trioleate ($TG_{18:1}$), and glycerol trilinoleate ($TG_{18:2}$). All were from NU-CHEK-PREP. β-Sitosterol was from Fluka (France).

Samples. One mature head (castrated individuals ready for the baking step) of each five agave taxa was analyzed, each of them coming from Mexican states included in the Denomination of Origin of mezcal (see **Table 1**). They were kept cold in sealed bags until brought to the laboratory, where they were stored at −20 °C before extractions. These were performed within no more than 3 months, since no variation of their lipid content along this time had been seen before in the laboratory.

Procedure. Samples were homogenized, cutting them off in small fractions that were then mixed. Random portions were chopped afterward with a Moulinex food processor. Three samples of 10 g of homogenized agave were subjected to drying in a vacuum oven for 45 h at 50 °C to determine the water content.

Modified Bligh−Dyer Lipid Extraction. Fifty grams of homogenized agave was analyzed in triplicate, employing the solvent volumes indicated in **Table 2**. By preliminary analyses that tested its absence in the samples, tridecanoic acid was used as an internal standard, adding 500 μL of a 16.2 mg/mL solution to the samples. These were then treated with the stated volume of CH_2Cl_2 (first addition) and CH_3OH for 2 min in a Waring blender. Then, the second addition of CH_2Cl_2 was performed and the system was blend for 30 s to finally add the water and blend for 30 s again. The extract was filtered with light suction through Whatman #5 filter paper on a Büchner funnel connected to a vacuum flask and then transferred into a 500 mL separatory funnel where the phases were allowed to separate. The residual tissue was reblended with 50 mL of CH_2Cl_2/CH_3OH 9:1 for 2 min, filtering the extract as before and pouring it into the separatory funnel to collect the total organic phase in a 250 mL round-bottom flask. The lipid extract was concentrated in a rotary evaporator and then transferred to a 10 mL volumetric flask, filling to the mark with dichloromethane.

Lipid Hydrolysis and Esterification. A 250 μL amount of lipid extract was placed in a 10 mL vial, and the solvent was evaporated under nitrogen stream. Afterward, 1.5 mL of 5% KOH in ethanol solution was added, and the system was heated at 80 °C for 30 min. Then, 1.5 mL of 10% HCl in ethanol solution was added, heating at 80 °C for 1 h. After the solution was cooled, FAEEs were extracted twice with 2 mL of hexane. The extract was washed with 2 mL of 4% $NaHCO_3$ aqueous solution and 1 mL of water, dried with anhydrous Na_2SO_4, and then concentrated under nitrogen stream to fill to the mark of a 1 mL volumetric flask. One microliter was injected into the gas chromatograph.

Derivatization for Total Simple Lipids Determination. The solvent of 1 mL of lipid extract placed in a 10 mL vial was removed by a nitrogen stream, and then, 1 mL of water, 2 mL of saturated NaCl aqueous solution, and 2 mL of 10% butanol in hexane were added, and the system was vortexed for 2 min. The organic phase was dried with anhydrous Na_2SO_4, taken to a volume of 2 mL in a volumetric

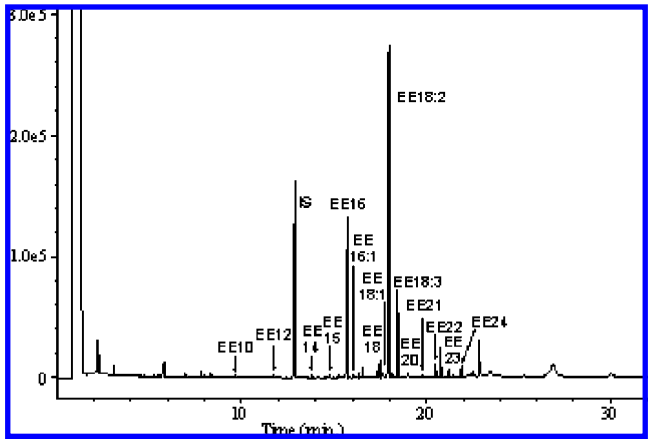

Figure 1. Example chromatogram of FAEEs of *A. salmiana* ssp. *crassispina*. IS: internal standard, EE13. Chromatographic conditions are as in the text.

flask, and transferred to a 4 mL vial. Here were added 100 μL of BSTFA, 100 μL of HMDS, and 50 μL of pyridine. The system was heated at 80 °C for 1 h after vortexing, and 1 μL of the so prepared extract was injected into the chromatograph.

Identification and Quantitation of FAEEs and Simple Lipids. Identification was achieved through comparison of relative retention times between sample peaks and lipid standard ones. The identity of FAEEs was also confirmed by gas chromatography−mass spectrometry. Quantitation was carried out by relative calibrations using EE13 as an internal standard for FAEEs and C13 for total simple lipid determinations; calibrations for FAEEs were made in high and low concentration ranges with EE16 as the calibration standard (CS) for the saturated homologue series and EE18:2 for the unsaturated ones. Calibration curves for simple lipids were built for the following compounds: hexanoic acid, 18-carbon atoms fatty acids (CS: C18:2), β-sitosterol, MGs (CS, $MG_{18:2}$), DGs (CS, $DG_{18:2}$), and TGs (CS, TG_{16}), with standards being subjected to derivatization with BSTFA, HMDS, and pyridine as in the total simple lipids determination. Method accuracy performance due to the derivatization procedures was evaluated through recovery tests of appropriate lipid standards spiked to the lipid extract of *A. cupreata*, which had shown the lowest amount of lipids in preliminary assays.

Chromatographic Conditions. An HP 5890 series II gas chromatograph with cold on-column injection system and flame ionization detection was used. A capillary column Quadrex Carbowax/BTR (30 m × 0.25 mm × 0.25 μm) was used for FAEEs analysis, with the following temperature program: initial oven temperature of 50 °C for 2 min, ramp of 10 °C/min to 250 °C, and final holding time of 10 min; the detector temperature was 250 °C, the injection port was kept 3 °C over the oven temperature (Oven Track On), and the carrier gas was H_2 at a constant flow rate of 2.44 mL/min. The same temperature program and column were employed in the gas chromatography−mass spectrometry analysis to confirm FAEEs identity with an HP 5890 gas chromatograph equipped with a split/splitless injector and coupled to an HP 5971 mass selective detector operated in full scan mode (70 eV electron impact ionization). The injection port temperature was set at 250 °C, while the transfer line and ion source temperatures were 280 and 175 °C, respectively. The total simple lipid analysis was done with a capillary column ZB-5HT Zebron (15 m × 0.25 mm × 0.10 μm) using the cold on-column gas chromatograph. In this case, the initial oven temperature was 45 °C for 1.5 min, then programming at 20 °C/min to 250 °C and immediately at 10 °C/min to 380 °C, holding there for 10.25 min. The injection port was kept 3 °C over the oven temperature (Oven Track On), and the carrier gas was H_2 at a flow rate of 2.2 mL/min.

RESULTS AND DISCUSSION

The results of water content determination of the homogenized agave tissues were as follows: *A. salmiana* ssp. *crassispina* 66.88 ± 0.27%, *A. angustifolia* 85.25 ± 0.26%, *A. cupreata* 61.85 ± 0.17%, *A. karwinskii* 66.22 ± 0.15%, and *A. salmiana* var. *salmiana* 73.09 ± 0.14%. Given these results, the solvent volumes pointed out in **Table 2** were calculated for having a first $CH_2Cl_2/CH_3OH/H_2O$ ratio of 1:2:0.8, necessary for a ternary monophasic system in the first dichloromethane addition and a ratio of 2:2:1.8 in the second one to get a biphasic system where the organic, lower phase would contain the extracted lipids (*16*, *17*). An improvement in the extraction efficiency using dichloromethane in the original Bligh−Dyer method had been seen previously (*13*), probably due to the lightly increased polarity of the extraction system, which could penetrate easier in the wet agave tissue and so break the lipid associations with other components in the vegetal matrix.

Fatty Acid Profiles. Sixteen fatty acids were identified as ethyl esters. All of them were present in the five analyzed agaves and went from the shortest EE10 to the longest EE24; neither EE11 nor EE19 was identified in this series in any of the taxa under study. **Figure 1** shows an example chromatogram of FAEEs of the agaves, pointing out EE18:2 as the largest peak, followed by EE16. The minimum resolution achieved between peaks EE18 and EE18:1 and between EE16 and EE16:1 in all

Table 3. Fatty Acid Concentrations in the Agaves

	agave									
	A. salmiana ssp. *crassispina*		*A. angustifolia*		*A. cupreata*		*A. karwinskii*		*A. salmiana* var.*salmiana*	
acid	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)
C10	0.40	5.7	0.11	4.3	0.23	7.0	0.32	3.4	0.42	3.1
C12	1.20	1.5	0.65	3.6	0.73	2.8	1.35	5.8	1.28	2.8
C14	1.87	1.6	1.36	3.6	1.65	1.3	2.77	3.3	3.52	3.8
C15	3.04	2.5	2.65	2.4	1.84	0.68	6.76	0.87	2.23	6.0
C16	130	1.3	163	1.7	128	1.6	210	0.51	172	4.4
C16:1	1.63	1.6	0.96	9.8	0.85	2.2	3.82	2.4	4.96	1.8
C17	7.06	2.3	3.55	2.4	2.36	1.5	6.44	1.4	3.67	3.9
C18	10.6	1.4	9.64	1.1	9.94	0.83	13.7	1.2	17.0	4.2
C18:1	14.1	0.5	30.5	2.1	59.4	1.2	155	2.8	110	4.2
C18:2	326	2.2	287	3.2	137	1.4	459	0.42	443	2.8
C18:3	70.0	4.3	39.4	4.2	86.6	1.5	108	1.8	50.0	3.8
C20	4.16	2.3	1.75	8.3	2.88	0.36	4.29	2.9	4.69	4.3
C21	2.29	1.8	0.56	1.4	0.97	4.7	1.07	8.8	1.58	4.1
C22	10.9	1.7	9.61	3.6	8.48	2.8	12.7	3.7	10.3	1.6
C23	7.72	4.0	4.14	4.7	4.86	4.3	5.39	3.3	7.62	1.1
C24	9.57	3.8	18.8	6.5	14.8	3.3	18.2	2.9	15.4	4.8
total	601	1.4	574	1.9	461	0.76	1008	0.57	849	2.0

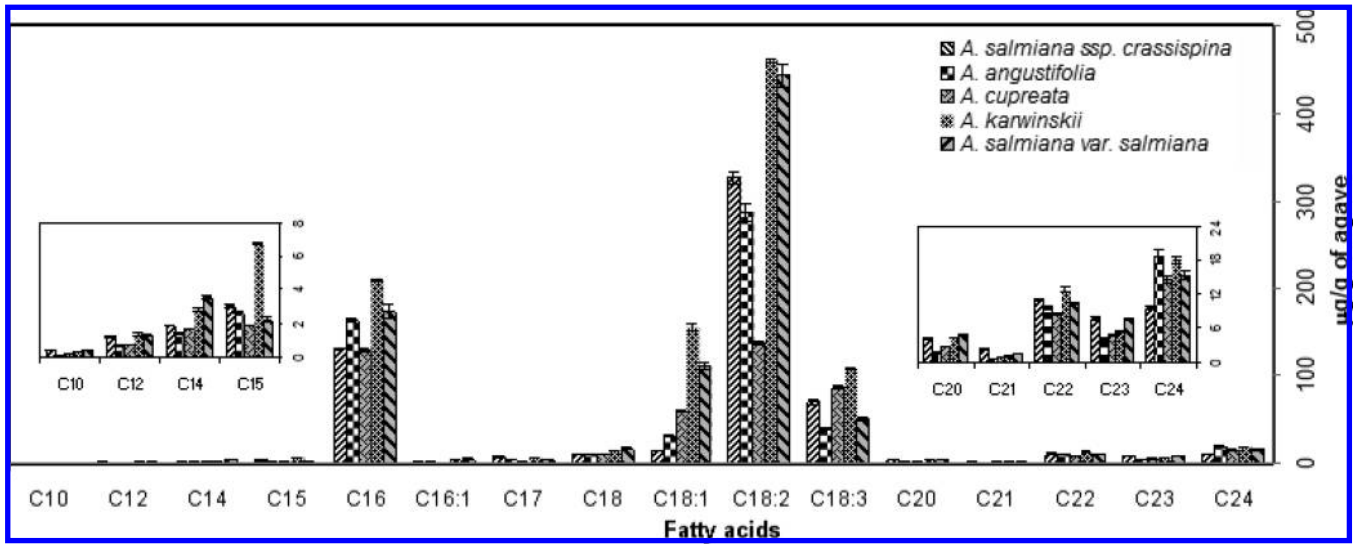

Figure 2. Comparison of fatty acid profiles of the five agaves.

of the analyses was 1.6 and 2.4, respectively, which means a practically complete separation between these near-eluting peaks.

Fatty acid concentrations are indicated in **Table 3**. As it can be seen, the method precision was good, having coefficients of variation (CV) less than 10% in all cases and even less than 5% in the main. **Figure 2** presents the general comparison of fatty acid profiles of the agaves; the higher content in palmitic acid (C16) and the unsaturated 18-carbon atoms fatty acids, especially linoleic (C18:2), is clearly observed. The sample with the highest concentration of fatty acids was *A. karwinskii*, followed in decreasing order by *A. salmiana* var. *salmiana*, *A. salmiana* ssp. *crassispina*, *A. angustifolia*, and *A. cupreata*. Capric acid (C10) was the one with the lowest concentration in all cases, and it was *A. angustifolia* that presented the lowest concentrations of the shorter (C10−C14) fatty acids identified. Apart from the major compounds (C16 and unsaturated C18s), whose concentration reached 459 μg/g in *A. karwinskii* for C18:2, the rest of the acids had concentrations less than 20 μg/g.

Fatty acids form a minor yet important group in the alcoholic beverages, giving specific odor and taste profiles to each drink. Fatty acids C2−C16 in the agave beverage tequila (*18*, *19*) have been identified, which can provide cheesy, oily, and musty notes. Even more important are the short and middle chain FAEEs that have pleasant aromas and low odor thresholds. For example, EE10 has a sweet, fruity, winey-cognac odor with 0.51 ppm threshold in wine; EE16 keeps the sweet odor with a waxy note and a >2 ppm threshold in water, and the longer FAEEs present oily and fatty flavors with increasing thresholds (*15*). Although they are not the ones with the highest concentrations, FAEEs are generally the most numerous organoleptic compounds in alcoholic beverages (*20*, *21*), probably due to the ethanol content (*22*). These compounds have been detected in mezcal and tequila, from EE2 to EE18 (*11*, *18*, *19*, *23*, *24*). From these considerations, a correlation between fatty acids and FAEEs content in mezcals from different producers and regions included in the Denomination of Origin and the corresponding raw material (agave) employed could be expected. A differentiation between the two types of mezcal, 100% agave and "mixto" (which uses less proportion of agave), could even be possible on the basis of their FAEEs content. These issues are to be explored.

The method accuracy from the derivatization step in the FAEEs analysis was evaluated through recovery tests. **Table 4** shows that the percent recovery of the compounds spiked to a lipid extract was in general within 100 ± 5%, although a little higher for EE20 and EE24, probably due to a differential extraction with respect to the internal standard.

Table 4. Spike Recovery Results in the FAEEs Analysis

spike	spiked FAEE concentration (ppm)	spiked FAEE concentration found (ppm)	spike recovery (%)
TG_{10}	0.247	0.243	98.4 ± 2.5
MG_{14} TG_{14}	2.57	2.64	103 ± 0.12
C16 DG_{16} TG_{16}	110	112	102 ± 0.20
C16:1	4.46	4.67	105 ± 0.52
C18 DG_{18}	10.3	10.5	102 ± 0.28
$TG_{18:1}$ C18:1	102	97.9	96 ± 0.41
$MG_{18:2}$ $DG_{18:2}$ $TG_{18:2}$	291	283	97.3 ± 0.21
DG_{20}	1.96	2.13	109 ± 1.9
C24	6.39	6.82	107 ± 1.2

Exploring the closeness between the fatty acid profiles of the five studied taxa was done with principal component and cluster analyses using Statgraphics Plus 5.1. In the principal component analysis (PCA), shown in **Figure 3**, the first component explained 53.88% of the variance and allowed us to observe a distribution of the taxa into two groups: *A. angustifolia* and *A. cupreata* (the two species with the lowest content of fatty acids) on the left side and *A. salmiana* ssp. *crassispina*, *A. salmiana* var. *salmiana*, and *A. karwinskii* on the right. There was not an apparent subgroup within the latter along the second component, which accounted for 25.69% of variance. However, cluster analysis revealed a major relationship between *A. salmiana* var. *salmiana* and *A. karwinskii*.

The corresponding dendrogram (**Figure 4**) agreed with the PCA results and pointed out to *A. angustifolia* and *A. cupreata* as the taxa with the closest similarity. It is noteworthy that the two species from the same state, *A. angustifolia* and *A. karwinskii*, from Oaxaca, were arranged in different clusters, which could be due either to differences in growing conditions within the same state or that the fatty acid profile is more correlated with the taxon itself than with the corresponding

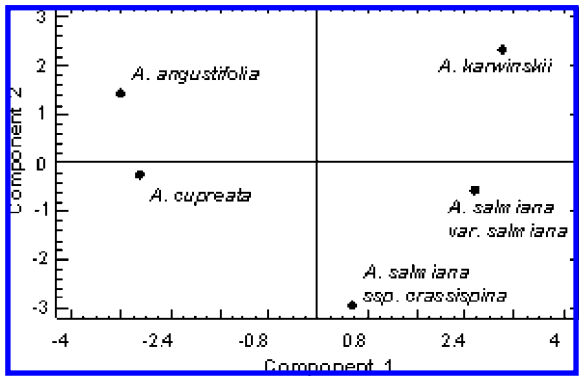

Figure 3. Principal component analysis based on the fatty acid profiles of the agaves.

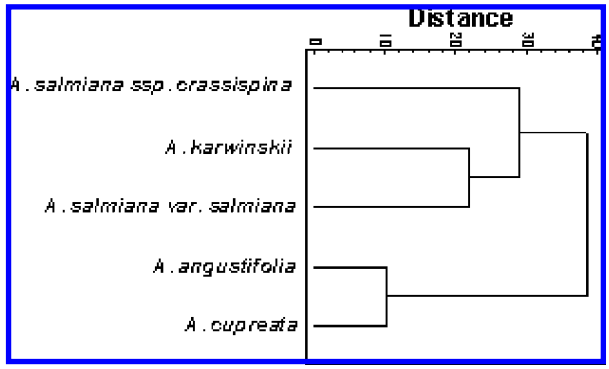

Figure 4. Dendrogram from fatty acid profiles showing relatedness among primary *Agave* taxa used to produce mezcal. It was obtained by the average linkage method using the squared Euclidean distance.

region of cultivation. Moreover, the two taxa of the species *salmiana* did not present the major similarity within their subgroup, in opposition to what could be expected. According to these results, it is thought that mezcals from the analyzed species *A. angustifolia* from Santiago Matatlán, Oaxaca, and *A. cupreata* from Chilapa, Guerrero, could have close fatty acid and FAEEs compositions, but this aspect as well as the influence of the production processes and the variation of fatty acid profiles between individuals and between populations of agave is still to be investigated.

Simple Lipid Profiles. Free fatty acids C16 and C18s, β-sitosterol, and groups of MGs, DGs, and TGs were identified in each agave in the total simple lipid analysis. **Figure 5** shows the corresponding chromatograms. In all cases but in *A. angustifolia*, a total concentration of TGs higher than of DGs was found. Peaks on the left of β-sitosterol are presumably other phytosterols: stigmasterol and campesterol, commonly found in plants (*25*). These plant sterols are known for their cholesterol-lowering effects (*26*, *27*), and β-sitosterol has also been cited for the treatment of benign prostatic hyperplasia (*28*). They are likely to be present in a Mexican beverage of agave juice with local consumption known as "aguamiel" and in its fermented form, the "pulque". Adjacent peaks to C16 in *A. angustifolia* were identified as palmitic and linoleic methyl esters, ME16 and ME18:2, appearing as much smaller peaks in the other samples. Also, *A. salmiana* var. *salmiana* presented peaks of low molar mass DGs, proportionally larger in this case in comparison with their occurrence in the rest of the agaves.

A variation in the distribution of acylglycerols in each case is noted, depending upon the carbon number, but in general, most larger peaks appear in the zone of higher molar masses due to acylglycerols with C16 and C18 chains. **Table 5** lists

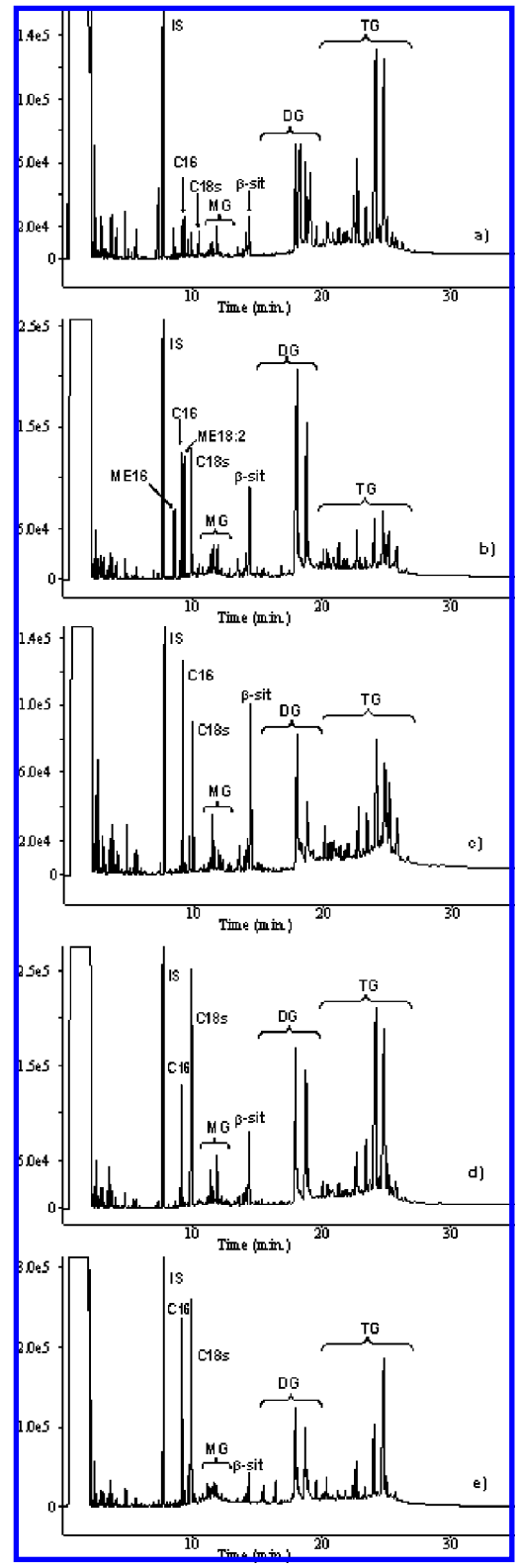

Figure 5. Chromatograms of total simple lipid analysis of (**a**) *A. salmiana* ssp. *crassispina*, (**b**) *A. angustifolia*, (**c**) *A. cupreata*, (**d**) *A. karwinskii*, and (**e**) *A. salmiana* var. *salmiana*. IS: internal standard, C13; β-sit, β-sitosterol. Chromatographic conditions and other abbreviations are as in the text.

the lipid concentrations found in the agaves. Total simple lipid concentrations found ranged from 459 to 992 μg/g of agave, which makes the plant an important source of these compounds, taking into account that some tons of agave piñas are typically

Table 5. Simple Lipid Concentrations in the Agaves

	agave									
	A. salmiana ssp. *crassispina*		*A. angustifolia*		*A. cupreata*		*A. karwinskii*		*A. salmiana* var. *salmiana*	
group	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)	μg/g	CV (%)
C16	4.77	1.6	18.7	5.7	16.1	1.5	21.2	5.7	41.7	6.9
C18s	4.04	6.4	24.9	5.1	18.2	3.2	60.0	9.5	81.8	3.7
β-sitosterol	7.88	1.0	25.4	6.4	23.8	3.1	27.7	1.6	11.7	9.3
MG	16.8	6.5	49.6	4.4	22.9	7.6	51.1	3.3	57.5	4.5
DG	237	3.3	244	8.0	109	7.0	267	3.8	257	5.0
TG	426	4.8	223	4.6	268	4.4	565	3.4	422	1.9
total	696	3.1	586	3.8	459	3.1	992	2.3	872	1.8

Table 6. Spike Recoveries in the Total Simple Lipid Analysis

spike	total spike concentration (ppm)	spike concentration found (ppm)	spike recovery (%)
C16	25.0	25.3	101 ± 2.5
C18s	23.5	24.4	104 ± 2.1
β-sitosterol	33.9	34.8	103 ± 2.7
MG			
MG_{16}	38.6	39.9	103 ± 2.9
$MG_{18:2}$			
DG			
DG_{16}	148	146	98.6 ± 1.3
$DG_{18:2}$			
TG			
TG_{16}	250	248	99.2 ± 1.2
$TG_{18:2}$			

employed in the cooking step of the production cycle of mezcal. CVs were in all cases less than 10%.

Table 5 clearly shows the predominance of TGs in most of the samples, constituting from 48 to 61% of total simple lipids, although in *A. angustifolia* the proportion of TGs (38 ± 2.3%) was similar to the one of DGs (42 ± 4%), as had been foreseen from the chromatogram. In accordance with the total fatty acid concentrations, the following decreasing order of total simple lipid levels is observed: *A. karwinskii*, *A. salmiana* var. *salmiana*, *A. salmiana* ssp. *crassispina*, *A. angustifolia*, and *A. cupreata*. The latter could provide a smaller amount of fatty acids and FAEEs to mezcal, but as mentioned before, its concentration in the samples depends upon the acid of concern. The evaluation of accuracy from the derivatization process in the total simple lipid analysis showed spike recoveries of 100 ± 5% in all cases (**Table 6**), which indicates an adequate lipid extract pretreatment and quantification procedure.

Acylglycerols are not found as such in mezcal but as fatty acids and their corresponding ethyl esters. It is important to mention that agave is not the only origin of these compounds in the spirit; other factors related with the production process like the cooking, fermentation, and distillation steps probably also take part (*20*, *29*, *30*). It remains as a next stage in the research to study the variation of simple lipid concentrations along the production process to the end product to establish more accurately the role of the agave as the source of these compounds.

As was observed, the analytical methods applied had good repeatability for these vegetal matrixes, offering CV less than 10% in all cases. Overall, they presented adequate spike recoveries in the FAEEs and total simple lipid analyses. The methodology is intended to be applied for further study of the lipid composition of plants within agave populations and regions of Mexico holding the Denomination of Origin 'Mezcal'. Also, our group is working on the characterization of other compound classes like terpenes, furans, aldehydes, etc. in the plant.

LITERATURE CITED

(1) Aguirre, J.; Charcas, H.; Flores, J. *El Maguey Mezcalero Potosino*; Universidad Autónoma de San Luis Potosí y Consejo Potosino de Ciencia y Tecnología: San Luis Potosí, Mexico, 2001; p 87.

(2) Granados, D. *Los Agaves en México*; Universidad Autónoma de Chapingo: Mexico, 1993; pp 11−59.

(3) Nobel, P. *Los Incomparables Agaves y Cactos*; Trillas: Mexico, 1998; pp 11−58.

(4) Gentry, H. *Agaves of Continental North America*; University of Arizona Press: Tucson, AZ, 1982; p 670.

(5) Eguiarte, L.; Souza, V.; Silva-Montellano, A. Evolución de la familia Agavaceae. *Bol. Soc. Bot. México* **2000**, *66*, 131–150.

(6) García-Mendoza, A. Distribution of Agave (Agavaceae) in Mexico. *Cact. Succ. J. (U.S.)* **2002**, *74*, 177–186.

(7) Declaración General de la Protección a la Denominación de Origen 'Mezcal'; Diario Oficial de la Federación: Mexico, D.F., November 28, 1994.

(8) Ley de la Propiedad Industrial, Título Quinto, Capítulo I; Diario Oficial de la Federación: Mexico, D.F., January 25, 2006.

(9) Eguiarte, L.; Souza, V. Historia natural del Agave y sus parientes: Evolución y ecología. In *En lo Ancestral Hay Futuro: Del Tequila, los Mezcales y Otros Agaves*; Colunga-García, P., Larqué, A., Eguiarte, E., Zizumbo-Villareal, D., Eds.; Centro de Investigación Científica de Yucatán: México, 2007; pp 3−21.

(10) Mexican Official Norm NOM-070-SCFI-1994. Bebidas Alcohólicas-Mezcal-Especificaciones; Diario Oficial de la Federación: Mexico, D.F., June 12, 1997.

(11) De León-Rodríguez, A.; Escalante-Minakata, P.; González-Hernández, L.; Barba, A.; López, M. Characterization of volatile compounds of mezcal, an ethnic alcoholic beverage obtained from *Agave salmiana*. *J. Agric. Food Chem.* **2006**, *54*, 1337–1341.

(12) Lachenmeier, D. W.; Sohnius, E.-M.; Attig, R.; Lopez, M. G. Quantification of selected volatile constituents and anions in mexican Agave spirits (Tequila, Mezcal, Sotol, Bacanora). *J. Agric. Food Chem.* **2006**, *54*, 3911–3915.

(13) Peña-Álvarez, A.; Díaz, L.; Medina, A.; Labastida, C.; Capella, S.; Vera, L. Characterization of three Agave species by gas chromatography and solid-phase microextraction-gas chromatography-mass spectrometry. *J. Chromatogr. A* **2004**, *1027*, 131–136.

(14) Michel-Cuello, C.; Juárez-Flores, B. I.; Aguirre-Rivera, J. R.; Pinos-Rodrìguez, J. M. Quantitative characterization of nonstructural carbohydrates of mezcal agave *Agave salmiana* Otto ex Salm-Dick. *J. Agric. Food Chem.* **2008**, *56*, 5753–5857.

(15) Leffingwell, J. C. *FLAVOR-BASE version 2007*; Leffingwell & Associates: Canton, GA, 1989−2007.

(16) Bligh, E.; Dyer, W. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.

(17) Smedes, F.; Thomansen, T. Evaluation of the Bligh & Dyer lipid determination method. *Mar. Pollut. Bull.* **1996**, *32*, 681–688.

(18) López, M. Tequila aroma. In *Flavor Chemistry of Ethnic Foods*; Shahidi, F., Ho, C., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 211−217.

(19) Peppard, T.; Benn, S. Characterization of tequila flavor by instrumental and sensory analysis. *J. Agric. Food. Chem*. **1996**, *44*, 557–566.
(20) Nykänen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic*. **1986**, *37*, 84–96.
(21) Russell, I. Yeast. In *Handbook of Brewing*, 2nd ed.; Priest, F., Stewart, G., Eds.; Taylor and Francis: Boca Raton, FL, 2006; pp 308−310.
(22) Engan, S. Beer composition: Volatile substances. In *Food Science and Technology*; Pollock, J. R. A., Ed; Academic Press: Great Britain, 1981; Vol. 2, pp 93−157.
(23) Vallejo-Cordoba, B.; González-Córdova, A.; Estrada-Montoya, M. Tequila volatile characterization and ethyl ester determination by solid phase microextraction gas chromatography/mass spectrometry analysis. *J. Agric. Food Chem*. **2004**, *52*, 5567–5571.
(24) Capella, S.; Covarrubias, M. Correlación de compuestos volátiles del tequila con su origen. *Bebidas Mexicanas*; December 2006/January 2007; pp 10−17.
(25) Heldt, H. *Plant Biochemistry*, 3rd ed.; Elsevier Academic Press: Burlington, MA, 2005; pp 363−372.
(26) Shin, M. J.; Rim, S. J.; Jang, Y.; Choi, D.; Kang, S. M.; Cho, S. Y.; Kim, S. S.; Kim, D. K.; Song, K.; Chung, N. The cholesterol-lowering effect of plant sterol-containing beverage in hypercholesterolemic subjects with low cholesterol intake. *Nutr. Res. (N.Y.)* **2003**, *23*, 489–496.
(27) Thompson, G. R.; Grundy, S. M. History and development of plant sterol and stanol esters for cholesterol-lowering purposes. *Am. J. Cardiol*. **2005**, *96* (1, Suppl. 1), S3–S9.
(28) Wilt, T. J.; MacDonald, R.; Ishani, A. Beta-sitosterol for the treatment of benign prostatic hyperplasia. *BJU Int*. **1999**, *83*, 976–983.
(29) Antonelli, A.; Castellari, L.; Zambonelli, C.; Carnaccini, A. Yeast influence on volatile composition of wines. *J. Agric. Food Chem*. **1999**, *47*, 1139–1144.
(30) López, M.; Mancilla-Margalli, N. Generation of Maillard compounds from inulin during the thermal processing of *Agave tequilana* Weber var. azul. *J. Agric. Food Chem*. **2002**, *50*, 806–812.

Received for review July 14, 2008. Revised manuscript received November 15, 2008. Accepted November 17, 2008. We thank DGAPA for the financial support (project PAPIIT IN207006-2).

JF802141D