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Characterization of Volatile Compounds of Mezcal, an Ethnic Alcoholic Beverage Obtained from *Agave salmiana*

ANTONIO DE LEÓN-RODRÍGUEZ,*[,†] LIDIA GONZÁLEZ-HERNÁNDEZ,[†] ANA P. BARBA DE LA ROSA,[†] PILAR ESCALANTE-MINAKATA,[†] AND MERCEDES G. LÓPEZ[‡]

División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, Apartado Postal 3-74 Tangamanga, 78231 San Luis Potosí, S.L.P., Mexico, and Centro de Investigación y de Estudios Avanzados del IPN-Unidad Irapuato, Departamento de Biotecnología y Bioquímica, Irapuato, Gto. 36500, Mexico

Commercial mezcals (white, white with worm, rested, rested with worm, and aged) produced from *Agave salmiana* were analyzed by solid-phase microextraction−gas chromatography−mass spectrometry (SPME−GC−MS). Thirty-seven compounds were identified, and nine of them were classified as major compounds of mezcal (MCM). Saturated alcohols, ethyl acetate, ethyl 2-hydroxypropanoate, and acetic acid form the MCM group. Minor compounds of mezcal group include other alcohols, aldehydes, ketones, large chain ethyl esters, organic acids, furans, terpenes, alkenes, and alkynes. Most of the compounds found in mezcals in this study are similar to those present in tequilas and other alcoholic beverages. However, mezcals contain unique compounds such as limonene and pentyl butanoate, which can be used as markers for the authenticity of mezcal produced from *A. salmiana.*

KEYWORDS: Mezcal; *Agave*; tequila; ethanol; SPME; terpenes; alcohols

INTRODUCTION

Mezcal is a Mexican alcoholic beverage obtained by artisan fermentation and distillation from *Agave* syrup. Species of *Agave* plants such as *A. salmiana*, *A. angustifolia*, and *A. potatorum* are used as raw materials (*1*). However, only the wild-type *A. salmiana* is used for the production of mezcal in the Mexican altiplano, whereas *A. angustifolia* and *A. potatorum* are used in the south of Mexico (*1*, *2*). Mezcals are beverages related to tequila (produced from *A. tequilana*). A full description of both processes and differences between mezcal and tequila has been published elsewhere (*2*−*4*).

Mezcals have been classified into three types based on the aging process after distillation. White mezcal is bottled just after distillation; rested mezcal is aged from 2 to 6 months in oak casks, whereas aged mezcal must be aged up to 12 months (*1*). Only the white and rested mezcals are conditioned with one to four larvae of *Agave* worms. Thus, mezcal is currently named “the worm’s beverage”.

The organoleptic properties and the bouquet of alcoholic beverages such as tequila, wine, and others are determined by the composition of alcohols mixture, esters, and other compounds. Principally, alcohols with three or more carbons and ethyl esters are the major agents responsible for the aroma (*5*−*10*). Microorganisms produce these compounds during the fermentation, although there are others coming from the raw material such as terpenes and large chain fatty acids (*6*). There exist some reports describing the components of tequilas (*7*−*10*); however, there is little information on the mezcal composition produced from *A. salmiana*, and only few reports of mezcal from *A. potatorum* and *A. angustifolia* are available (*11*, *12*).

The techniques reported for analysis of alcoholic beverages include the concentration of components by micro distillation, liquid−liquid extraction, and solid-phase microextraction (SPME) (*8*−*11*). SPME is a current sampling technique recommended for the analysis of alcoholic beverages because it is solvent-free and sample handling is minimized (*10*−*15*). The goal of this work was to characterize the volatile compounds of mezcals obtained from *A. salmiana* through GC and SPME−GC−MS techniques.

MATERIALS AND METHODS

Chemicals and Mezcal Samples. All chemicals used as standards with purity up to 99.0% GC grade were purchased from Sigma-Aldrich (St. Louis, MO). Sixteen mezcal brands were analyzed: four white mezcals, three white with worm, three rested, three rested with worm, and three aged mezcals. They were kindly provided by the Association of Mezcal Producers of San Luis Potosi State, Mexico.

Major Compounds of Mezcal. The major compounds of mezcal (MCM) are the substances with concentration larger than 10 mg/L, and they are detected and quantified by GC using direct injection of the raw samples. For the quantitation of MCM, 1 mL of 2-pentanol at

* Author to whom correspondence should be addressed [telephone +52 (444) 8342000; fax +52 (444) 8342010; e-mail aleonr@ipicyt.edu.mx].

[†] Instituto Potosino de Investigación Científica y Tecnológica.

[‡] Centro de Investigación y de Estudios Avanzados del IPN-Unidad Irapuato.

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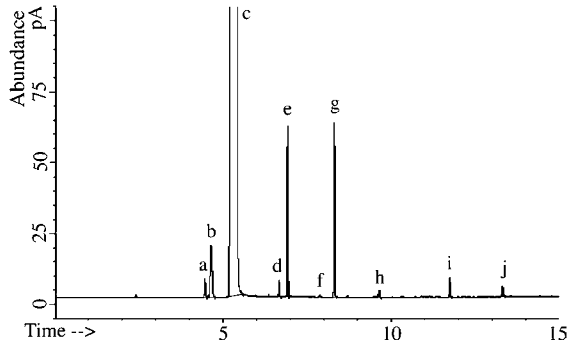

Figure 1. Chromatogram of major compounds found in rested mezcal with worm. Refer to **Table 1** for the retention times of ethyl acetate (**a**), metanol (**b**), ethanol (**c**), 2-butanol (**d**), *n*-propanol (**e**), 2-methyl-propanol (**f**), 2/3 methyl-1-butanol (**h**), ethyl-2-hydroxypropanoate (**i**), and acetic acid (**j**). 2-Pentanol (**g**) was used as internal standard.

3.5 mg/mL as internal standard was added to 8 mL of each mezcal sample up to 10 mL with milliQ water. The samples were analyzed by injecting 1 μL in a gas chromatograph 6890N (Agilent technologies, Wilmington, DE) provided with an auto-sampler 7863 (Agilent technologies, Wilmington, DE) and a capillary column HP-Innowax (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness; Agilent technologies, Wilmington, DE). Helium was used as carrier gas at a flow rate of 1.5 mL/min, and the setting temperatures for the injector and flame ionization detector (FID) were 220 and 250 °C, respectively. The chromatographic conditions for the quantitation of MCM, excepting the ethanol, were a split relation of 10:1 and a temperature program of 35 °C for 2 min, increased at 10 °C/min to 210 °C, and maintained at this temperature to a final time of 20.5 min. The ethanol quantitation was done with a split relation of 25:1 and a temperature program of 35 °C for 2 min, increased at 10 °C/min to 80 °C, and maintained at this temperature to a final time of 15 min. All brands were analyzed in triplicates, and their medias were used to assess the average of compound concentration for each type of mezcal, and these values were compared as a function of aging. The compounds were identified by comparing the retention time of standards and by the NIST library of the MS.

The standard curves for quantitation of the MCM excepting ethanol were prepared in a 40% (v/v) ethanol solution, while standards for ethanol were prepared using distillated water. The calibration curves included a range of 10−1000 mg/L for each compound.

Minor Compounds of Mezcal. The minor compounds of mezcal are the substances detected after sample concentration by SPME followed by GC−MS analysis. Two mL of each mezcal was placed in 4 mL vials, and samples were incubated at room temperature with magnetic agitation at 100 rpm for 2 h using a SPME device (Supelco, Bellefonte, PA). Later, a SPME fiber of 65 μm de Carbowax/Divinylbenzene (CW/DVB) was exposed to the headspace by 60 min. The SPME fiber was immediately inserted in the GC injector in split-less mode for 1 min at 180 °C. The GC−MS analyses were carried out in a gas chromatograph HP 5890 (Hewelett-Packard) coupled to a HP 5971 mass selective detector (Hewelett-Packard) and using a HP-FFAP column (50 m $\times$ 0.32 mm, 0.5 μm thickness; Agilent technologies, Wilmington, DE). The chromatographic conditions were 40 °C for 3 min, increased at 3 °C/min to 120 °C, 6 °C/min to 200 °C, and maintained at this temperature to a final time of 60 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min, and the injector and detector temperatures were 180 and 230 °C, respectively. The MS ionization potential was 70 eV, transfer line temperature of 230 °C, and scan mode (50−700 *m/z*). The compounds were tentatively identified by comparing their mass spectra to those obtained in the NIST library of the MS database.

RESULTS AND DISCUSSION

Analysis of Major Compounds in Mezcal. The chromatogram for a rested mezcal with worms is shown in **Figure 1**. Chromatograms of other mezcals followed the same trend as those in **Figure 1**, although the peak heights were different for each case. The retention times for the major compounds and their concentrations are summarized in **Table 1**. The alcohols found were ethanol, methanol, *n*-propanol, 2-butanol, 2-methyl-propanol, and a mixture of 2-methyl-1-butanol and 3-methyl-1-butanol. Because these isomers can be solved only on specific chromatographic conditions, they are usually reported together as 2/3 methyl-1-butanol (*7, 9*). As expected, ethanol was the most abundant component in mezcal as a result of sugar biotransformation by the microorganisms during the fermentation. On the other hand, high alcohols such as 2-butanol, 2-methyl-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol are produced by the catabolism of amino acids, even aldehydes that are reduced by an alcohol dehydrogenase to their respective alcohols, although their specific production rates are lower than that attained for the ethanol (*16*). The production of methanol is inherent during alcoholic fermentation because it is produced from pectin and lignin of the vegetal-cell wall (*2*). However, because of the neurotoxic effect of methanol, the maximum concentration permitted is 300 mg/100 mL of anhydrous ethyl alcohol (approximately 1200 mg/L) (*1, 2*). For the mezcals tested, the highest concentration of methanol was 891 $\pm$ 46 mg/L in the rested mezcals. Other major compounds also found in the mezcals are acetic acid, ethyl acetate, and ethyl 2-hydroxypropanoate. For tequila, the major components reported were ethanol, 2/3 methyl-butanol, phenylethyl alcohol, acetic, decanoic, and dodecanoic acids (*7*). While for mezcals obtained from *A. angustifolia* the major components reported are the same as those reported in this work, in addition, furfural and 5-methyl-furfulaldehyde were detected. The major components reported for Sotol, an ethnic alcoholic beverage obtained from *Dasilyrion sp.*, include, besides furfural, naphthalene and 1-methyl-

Table 1. Concentrations (mg/L) of MCM Found in Different Kinds of Mezcals

Rt[a] (min)	compound	white[d] ($n = 4$)	white with worms ($n = 3$)	rested ($n = 3$)	rested with worms ($n = 3$)	aged ($n = 3$)
4.46	ethyl acetate	182 ± 11	103 ± 6	150 ± 12	113 ± 5	107 ± 10
4.63	methanol	816 ± 72	703 ± 89	891 ± 46	881 ± 38	834 ± 76
5.30	ethanol[b]	42 ± 1	39 ± 2	39 ± 2	42 ± 0	42 ± 5
6.66	2-butanol	61 ± 17	54 ± 19	66 ± 20	56 ± 6	ND
6.91	*n*-propanol	700 ± 108	728 ± 18	738 ± 87	615 ± 62	388 ± 26
7.87	2-methyl-propanol	17 ± 4	ND	ND	37 ± 4	48 ± 7
9.62	2/3-methyl-1-butanol	30 ± 7	26 ± 10	ND	17 ± 4	98 ± 19
11.72	ethyl 2-hydroxypropanoate	105 ± 13	101 ± 4	117 ± 15	109 ± 17	192 ± 32
13.26	acetic acid	169 ± 51	224 ± 48	207 ± 27	133 ± 5	219 ± 9
	high alcohols[c]	809 ± 122	808 ± 32	843 ± 64	876 ± 52	533 ± 51

[a] Rt = retention time in the HP-Innowax column. [b] Unit for ethanol concentration is %v/v. [c] Sum of alcohol with three or more carbons. [d] ND = not detectable, n = number of brands. Data represent the average ± standard deviation of each type of mezcal as described in Materials and Methods.

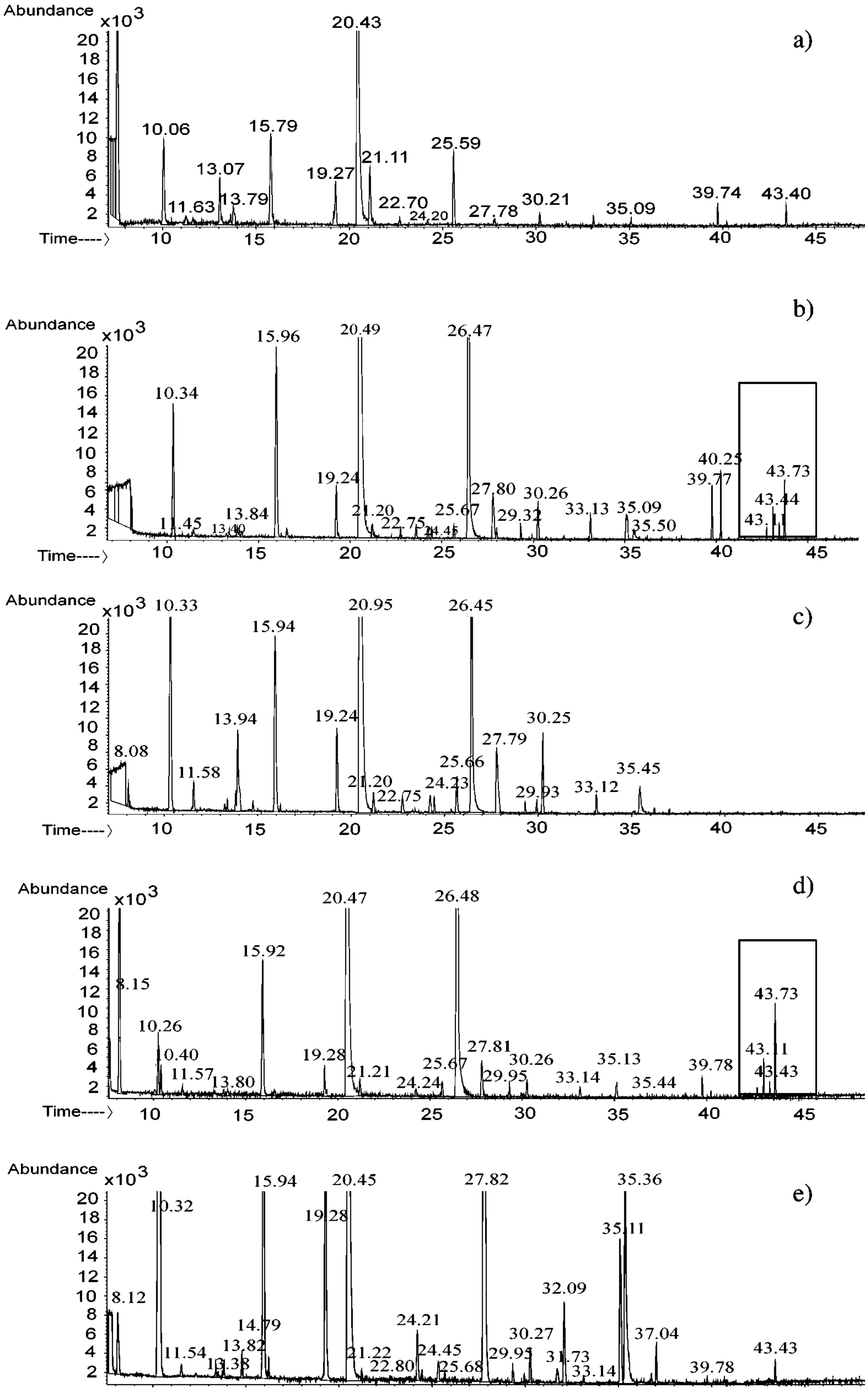

Figure 2. Chromatograms of five kinds of mezcals from *A. salmiana* analyzed by SPME–GC–MS: white (**a**), white with worms (**b**), rested (**c**), rested with worms (**d**), and aged (**e**). Frames show the compounds unique in mezcals with worm.

naphthalene (*11*). The phenylethyl alcohol, large chain fatty acids ethyl esters, furfural, 5-methyl-furfuraldehyde, and naphthalene are present in mezcals analyzed in this work but as minor components described below.

Significant differences were observed in the concentration of the MCM in the brands analyzed. The concentrations of ethyl acetate, methanol, ethanol, and acetic acid are similar in mezcals at different aging stages, while 2-methyl-propanol and ethyl 2-hydroxypropanoate in aged mezcals increased with aging. On the other hands, the concentration of 2-butanol, *n*-propanol, and high alcohols decreased possibly by esterification reactions during aging (*8*). These changes on concentration could be used

Table 2. Compounds Detected in Mezcals by SPME–GC–MS: (1) White, (2) White with Worms, (3) Rested, (4) Rested with Worms, and (5) Aged

Rt[a]	compound	mezcal kind
	Alcohols	
1.51	methanol	1,2,3,4,5
3.66	2-butanol	1,2,3,4,5
4.06	propanol	1,2,3,4,5
5.88	2-methyl-propanol	1,2,3,4,5
8.72	butanol	3,4,5
10.26	2/3-methyl-1-butanol	1,2,3,4,5
37.04	phenylethyl alcohol	3,4,5
42.11	6,9-pentadecadien-1-ol	2,4
42.75	3-hexen-1-ol	2,4
	Esters	
1.19	ethyl acetate	1,2,3,4,5
3.88	ethyl butanoate	1,2,3,4,5
6.66	ethyl pentanoate	1,2,3,4,5
10.4	ethyl hexanoate	1,2,3,4,5
13.9	pentyl butanoate	1,2,3,4,5
15.9	ethyl 2-hydroxypropanoate	1,2,3,4,5
19.28	ethyl octanoate	1,2,3,4,5
27.81	ethyl decanoate	1,2,3,4,5
35.1	ethyl dodecanoate	1,2,3,4,5
39.77	ethyl hexadecanoate	1,2,3,4,5
43.43	ethyl octadecanoate	1,2,3,4,5
	Aldehydes and Ketones	
1.22	acetaldehyde	1,2,3,4,5
13.07	3-hydroxy-2-butanone	1,2,3,4,5
	Acids	
20.4	acetic acid	1,2,3,4,5
24.2	propionic acid	1,2,3,4,5
32.12	pentanoic acid	2,5
35.44	hexanoic acid	1,2,3,4,5
	Furans	
10.02	2-pentylfuran	1,4
21.20	furfural	1,2,4,5
25.66	5-methyl-furfuraldehyde	1,2,4
	Terpenes	
8.15	limonene	1,2,3,4,5
29.96	α-terpinene	1,2,3,4,5
30.2	α-terpineol	1,2,3,4,5
	Others	
11.5	4-ethyl-1,2-dimethyl benzene	1,2,3,4,5
22.79	methyl-2-pentene	1,2,3,5
31.73	naphthalene	1,3,5
43.1	1,8-nonadiene	2,4
43.7	1-dodecyne	2,4

[a] Rt = retention time in the HP-FFAP column.

as markers for the classification of mezcals types. However, the analysis of many samples is necessary to establish valid concentration ranges to allow a correct classification.

Minor Compounds in Mezcal. Figure 2 shows the SPME−GC−MS analysis for each type of mezcal tested, and **Table 2** lists the 37 compounds identified. It is possible that other compounds are present at lower concentration and they were not identified in this work. Butanol and phenylethyl alcohol were detected in addition to the alcohols detected and were quantified as major components in rested and aged mezcals. Unsaturated alcohols such as 3-hexen-1-ol and 6,9-pentadecadien-1-ol were detected only in mezcals with worms.

An abundant group of ethyl esters of C_2−C_{18} and pentylbutanoate were detected in all mezcals. Interestingly, pentyl butanoate has not been detected in other alcoholic beverages such as tequila, sotol, or mezcal produced from *A. angustifolia* (*9*−*12*); thus this compound could be used as an authenticity marker for mezcal produced from *A. salmiana*. The ethyl esters are associated with the bouquet and pleasant fruity flavors, and they have been detected in other alcoholic beverages such as tequila, whiskey, cognac, and others (*7*−*15*). The concentration of large chain acid ethyl esters (C_8−C_{18}) in tequila increased with aging, and it has been proposed that their concentration may be used as an alternative for the classification of tequila types (*10*). Acetic, propionic, and hexanoic acids were found in all mezcals tested, while pentanoic acid was only found in white mezcal with worms and aged mezcals. Perhaps the others mezcals contain pentanoic acid, but its concentration was too low to be detected. Other chemical groups such as aldehydes, ketones, furans, terpenes, naphthalenes, alkenes, and alkynes were also detected. Despite the low concentration of minor compounds, their presence is relevant because they harmonically synergize to produce the characteristic mezcal aroma. For tequilas, the minor compounds reported are esters, acids, furans, ketones, terpens, and others (*7*). López and Guevara-Yáñez determined the volatiles of mezcal obtained from *A. angustifolia*, tequila, and sotol. The study showed that the most of the compounds are present in all of the beverages but there are few compounds such as nonanoic acid ethyl ester, 2-methylnaphthalene, and 2-acetylfuran that are unique in each beverage and they can be used as authenticity markers for mezcal (from *A. angustifolia*), sotol, and tequila, respectively (*11*). Interesting, these compounds were not found in the mezcals analyzed here.

Peña-Alvarez et al. (*6*) identified nine terpenes (α-phellandrene, α-terpinene, *p*-cimene, limonene, α-*trans*-ocimene, linalool, 4-terpineol, geraniol, and *trans*-nerolidol) in plants of *A. salmiana* (*6*). It has been reported that terpenes are liberated by *β*-glycosidases from yeasts during the fermentation process (*17*, *18*). Therefore, they can be found in alcoholic beverages and used as markers of authenticity. In our study, we detected only three terpenes: limonene, α-terpineol, and α-terpinene. The terpenes reported for tequila are lialoxide, linalool, linalyl propanoate, nerolidol, thymol, and terpineol (*7*, *9*); meanwhile, in plants of *Agave tequilana* up to 32 terpenes were found (*6*). It is possible that some terpenes are lost during the beverages production or their concentration is too low to be detected or identified in the commercial beverages.

Only mezcals with worms showed a group of compounds with retention time between 41 and 45 min; they are 6,9-pentadecadien-1-ol, 3-hexen-1-ol, 1,8-nonadiene, and 1-dodecine (**Figure 2**). Thus, it is possible that the 3-hexen-1-ol and the other unsaturated compounds come from the larvae. The 3-hexen-1-ol is commonly found in the green leaf alcohol and insects, and it has been reported that it displays a pheromonal activity in elephants and insects (*19*, *20*). According to this study, most of the compounds found in mezcals are similar to those reported for tequilas and other alcoholic beverages. However, mezcals produced from *A. salmiana* contain unique compounds such as limonene and pentyl butanoate, which can be used as markers for the authenticity.

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