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Characterization of Volatile Compounds from Ethnic *Agave* Alcoholic Beverages by Gas Chromatography-Mass Spectrometry

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Summary

Ethnic *Agave* alcoholic beverages such as raicilla, sisal, tequila, mezcal, bacanora, sotol and pulque have been analyzed by gas chromatography and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). There were 105 compounds identified, eleven were classified as major compounds and the others were classified as minor compounds. Seventeen minor compounds could be used as authenticity markers since they were beverage specific. Cluster analysis (CA) showed that *Agave* alcoholic beverages could be distinguished by multivariate analysis of major compounds; however, the analysis of minor compounds provided a better fingerprinting.

Key words: Agave, pulque, spirits, SPME, terpenes, alcohols

Introduction

Many countries have the denomination of origin and exclusive trademarks for local alcoholic beverages such as Scotland for whisky (*1*), France for cognac (*2*), Mexico for tequila and others (*3*). Mexico has several native plants of *Agave* genus and some of them are used for the production of ethnic *Agave* alcoholic beverages (*4*). For example, pulque is a nondistilled alcoholic beverage containing 4–6 % (by volume) alcohol and it is produced by the fermentation of *Agave* sap (aguamiel) obtained mainly from *A. mapisaga.* Distilled beverages with high alcohol fraction (30–45 %) are produced by fermentation and distilling of sugars from cooked *Agave* plants. The names of ethnic *Agave* alcoholic beverages depend on both the *Agave* species used as raw material and the geographic region of Mexico where they are cultivated. Tequila is produced from *A. tequilana* Weber var. *azul*, raicilla from *A. maximiliana,* sisal from *A. sisalana* and sotol from *Dasylirion wheeleri. A. angustifolia* Haw. is used to produce bacanora in the north and mezcal in the south of Mexico. Mezcal is also produced from *A. salmiana*, *A. potatorum* or *A. durangensis* in specific zones of Mexico (Fig. 1).

Volatile compounds determine the organoleptic properties and the bouquet of alcoholic beverages. Flavour is a combination of both aroma and taste, aroma being the perception of volatiles through mouth and olfactory system (*5*). Composition of tequila (*6*), mezcal (*7*), sotol and bacanora (*8*) has been reported previously. However, there are not exhaustive studies available on volatile compounds by gas chromatography (GC) coupled with mass spectrometry (MS) for other *Agave* alcoholic beverages such as sisal, raicilla, pulque and mezcals from different plants.

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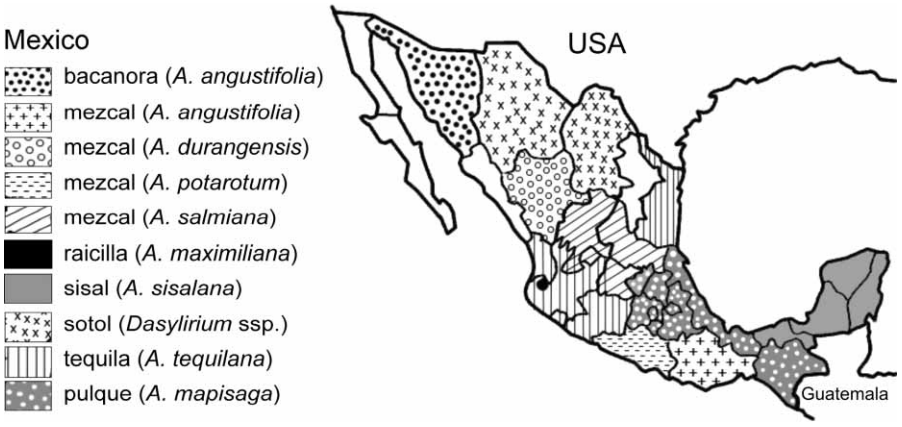

Fig. 1. Geographical location of *Agave* plants used for production of *Agave* alcoholic beverages in Mexico

The solid-phase microextraction (SPME) is a sampling technique currently recommended for the GC-MS analysis of alcoholic beverages since it is solvent-free and minimizes sample handling (*9,10*). The aim of this work is an extensive characterization of volatile compounds of different ethnic alcoholic beverages produced from *Agave* plants by GC and SPME-GC-MS techniques.

Materials and Methods

Chemicals and mezcal samples

Ethanol, methanol, propanol, 2-butanol, 2-pentanol, 2-methylpropanol, 2-methyl-1-butanol, acetic acid, ethyl acetate, and ethyl-2-hydroxypropanoate with purity of 99 % GC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). Certified beverages by the Mexican Ministry of Commerce and Industry were purchased in approved beverage stores, whereas pulque was purchased in bulk.

Gas chromatography

The quantitation of major compounds present in a concentration larger than 10 mg/L was performed in a gas chromatograph 6890N (Agilent Technologies Inc., Wilmington, DE, USA) provided with an autosampler 7863 (Agilent Technologies Inc., Wilmington, DE, USA) and a capillary column HP-Innowax (30 m×0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies Inc., Wilmington, DE, USA). 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 Agilent Technologies Inc., Wilmington, DE, USA) were 220 and 250 °C, respectively. The operative conditions and details on the calibration curves had been described elsewhere (*7*). Samples were analyzed in triplicate and the average concentration of each compound was used for our results.

Headspace SPME-GC-MS

The minor compounds are the substances detected after sample concentration by headspace solid-phase microextration (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS) analysis. The HS--SPME operation was carried out as described by De León-Rodríguez *et al*. (*7*) using separately an SPME orange fiber of 65 μm (Carbowax/divinylbenzene, CW/DVB) and an SPME black fiber of 65 μm (Carboxen/polydimethylsiloxane, CAR/PDMS). The SPME fibers were immediately inserted in the GC injector in splitless mode for 1 min at 180 °C. The GC-MS analyses were carried out in a gas chromatograph 6890N (Agilent Technologies Inc., Wilmington, DE, USA) coupled to an HP 5973N mass selective detector (Agilent Technologies Inc., Wilmington, DE, USA) and a DB-WAX column (30 m× 0.32 mm, 0.5 μm thickness; Agilent Technologies Inc., Wilmington, DE, USA). 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 for a final time of 60 min (*7*). 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 was 230 °C, and the scan mode was 50–700 *m/z*. The compounds were tentatively identified by comparing their mass spectra with those obtained in the NIST library of the MS database.

Cluster analysis

The cluster analysis (CA) for quantitative evaluation of major compounds was carried out by the nearest neighbor (single linkage) clustering method using Statgraphic Plus software ver. 5 (Statistical Graphic Corp., Herndon, VA, USA). The CA for minor compounds was carried out with the UPGMA (with Jaccard coefficient) clustering method using NTSysPc ver. 3.21 (*11*).

Results and Discussion

Analysis of major compounds

The chromatograms of the analyzed *Agave* alcoholic beverages are shown in Fig. 2 and the compounds and their respective concentrations are summarized in Table 1. The alcohols detected were methanol, ethanol, propanol, 2-butanol, 2-methylpropanol, 2-phenylethanol and the mixture of 2-methyl-1-butanol and 3-methyl-1-butanol. Since these isomers can be distinguished only under specific chromatographic conditions, they are reported together as 2-(3-)-methyl-1-butanol (*6,7*). Acetic acid,

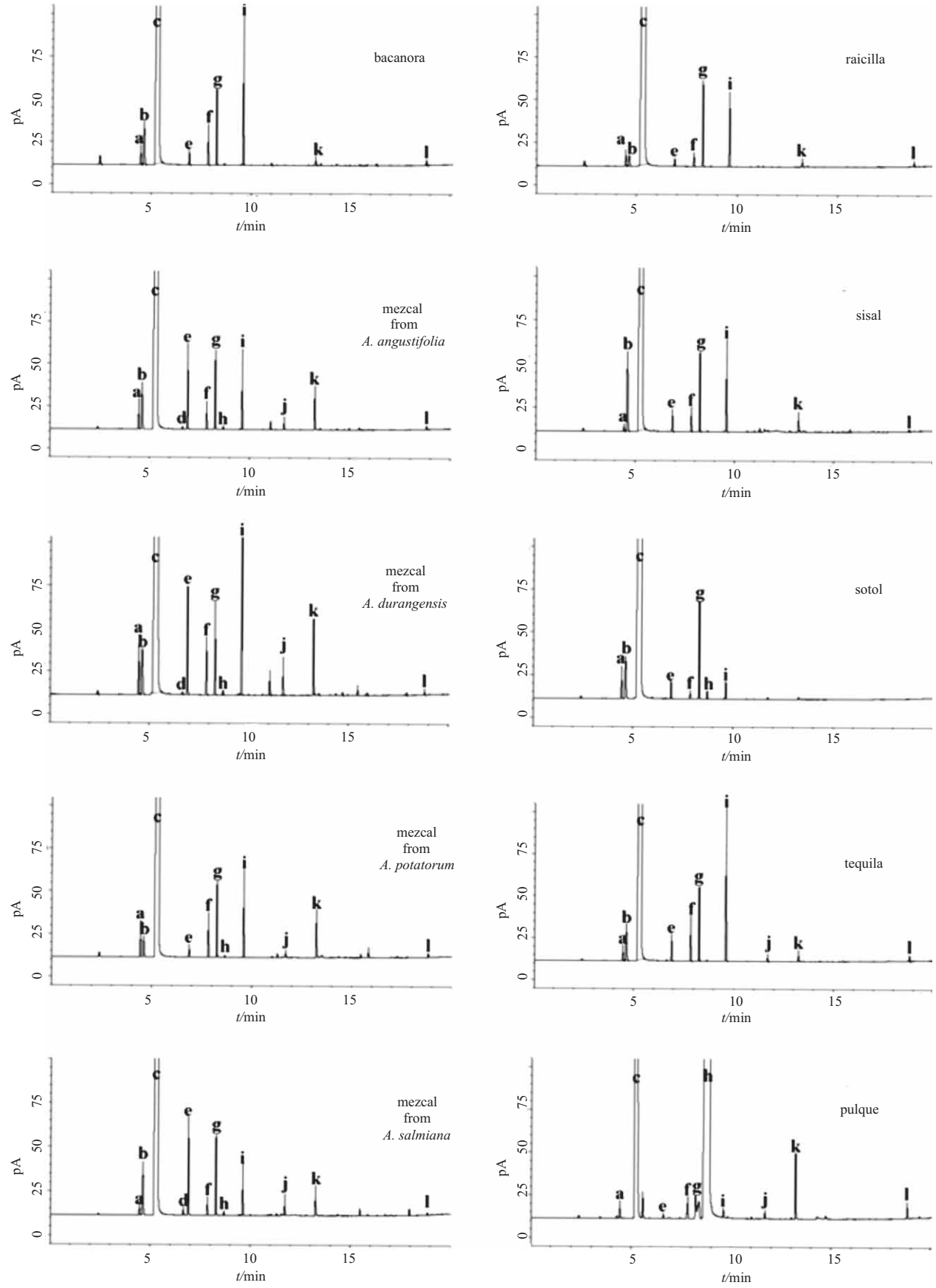

Fig. 2. Chromatograms of *Agave* alcoholic beverages analyzed by GC-FID, a=ethyl acetate, b=methanol, c=ethanol, d=2-butanol, e=propanol, f=2-methylpropanol, g=2-pentanol (internal standard), h=1-butanol, i=2-(3-)-methyl-1-butanol, j=ethyl-2-hydroxypropanoate, k=acetic acid, l=2-phenylethanol

Table 1. Concentration of major compounds found in alcoholic beverages produced from different species of *Agave* plants

RT/ min	Compound	γ/(mg/L)									
		Bacanora	Mezcal from *A. angustifolia*	Mezcal from *A. durangensis*	Mezcal from *A. potatorum*	Mezcal from *A. salmiana*	Raicilla	Sisal	Sotol	Tequila	Pulque
4.46	Ethyl acetate	284±5	395±3	474±6	492±1	170±0	166±1	100±2	323±2	221±2	270±5
4.63	Methanol	1065±2	1024±6	942±3	472±1	1162±4	217±3	1826±11	805±8	880±7	ND
5.30	Ethanol	339000	307700	370000	290000	307000	315000	307000	315000	362000	39000
6.66	2-Butanol	ND	21±0	17±0	ND	59±0	ND	ND	ND	ND	47±1
6.91	Propanol	111±0	612±2	580±1	97±0	708±2	41±1	163±1	99±1	208±8	ND
7.87	2-Methylpropanol	294±2	201±1	307±0	321±0	126±0	83±1	172±0	50±0	340±1	218±4
8.64	1-Butanol	ND	21±1	22±3	13±2	14±1	ND	ND	35±1	ND	ND
9.62	2-(3-)-Methyl-1-butanol	926±1	472±0	1064±2	589±1	307±1	350±3	545±1	83±0	1068±10	65±1
11.72	Ethyl-2-hydroxypropanoate	ND	124±2	265±3	73±1	189±2	ND	ND	ND	77±1	85±2
13.26	Acetic acid	207±2	939±6	1192±25	1042±29	638±8	160±6	441±55	ND	214±47	1530±19
18.82	2-Phenylethanol	23±0	14±1	18±0	17±0	10±2	16±1	9±0	ND	23±0.5	64±0
–	Higher alcohols*	1354±1	1339±4	2007±1	1037±2	1220±5	490±4	888±2	267±1	1638±13	394±4

RT=retention time in the HP-Innowax column; *sum of alcohol with three or more carbons; ND=not detected. Data represent the average±standard deviation as described in Materials and Methods section

ethyl acetate, and ethyl-2-hydroxypropanoate were also found in all alcoholic beverages. Important differences in the concentration of major compounds in each type of beverage were observed. The compounds marked as undetectable had concentration lower than 10 mg/L and they were referred to as minor compounds (described below). The major compounds are produced by the alcoholic fermentation (*12*,*13*). The compound 2-(3-)-methyl--1-butanol has a fruity aroma, and its presence is desired in alcoholic beverages (*12*,*13*). In our study, bacanora, mezcal (from *A. durangensis*) and tequila were the beverages with the highest concentration of 2-(3-)-methyl-1--butanol containing up to 900 mg/L, while sotol had the lowest concentration (83 mg/L). Propanol has an unpleasant aroma and its presence should be as low as possible. Mezcal from *A. salmiana* had the highest concentration of propanol of 708 mg/L, while raicilla had only 41 mg/L. For pulque, the concentrations of 2-(3-)-methyl--1-butanol and propanol were 65 mg/L and undetectable, respectively. The differences in the concentrations of major compounds between the alcoholic beverages could be attributed to the microorganism community during fermentation phase and distilling conditions as well.

Fig. 3 shows the cluster analysis (CA) for the *Agave* alcoholic beverages using the major compounds. The primary cluster included all distilled alcoholic beverages, whereas pulque was separate due to low concentration of ethanol. Mezcals were grouped in a subset, because they are regulated by the same rules as described by the Mexican Ministry of Commerce and Industry (*14*). A subsequent branch included sisal and in the last group were bacanora and tequila. CA showed that *Agave* alcoholic beverages could be distinguished by multivariate analysis of major compounds. Cardoso *et al.* (*15*) reported that cachaça and rum (two alcoholic beverages produced from sugar cane in Brazil and the Caribbean,

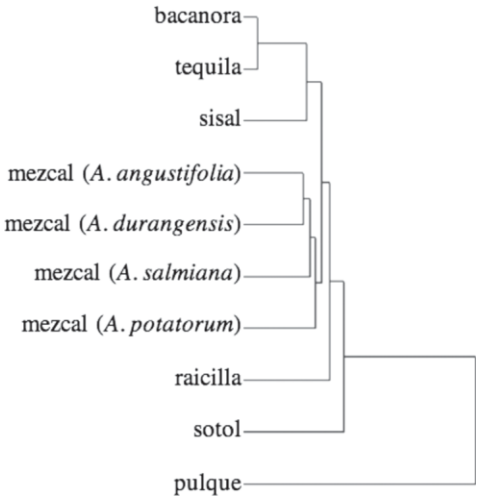

Fig. 3. Dendrogram of cluster analysis for alcoholic beverages produced from different species of *Agave* plants

respectively) could be distinguished by CA based on the concentration of higher alcohols (propanol, isobutanol and isopentanol) and metals. The content of higher alcohols has been used to certify Irish whiskey authenticity (*16*). The analysis of major compounds has been proposed as aging indicator of mezcal from *A. salmiana* (*7*). The concentration of alcohols, esters and fatty acids can be used to classify and differentiate the origin of a variety of wines (*17*).

Analysis of minor compounds by HS-SPME-GC-MS

The HS-SPME-GC-MS analysis for tequila using orange and black fibers is shown in Fig. 4. Compounds such as 3-furaldehyde (retention time, RT=21.20), 1-(2--furanyl)-ethanone (RT=22.86) and others showed a

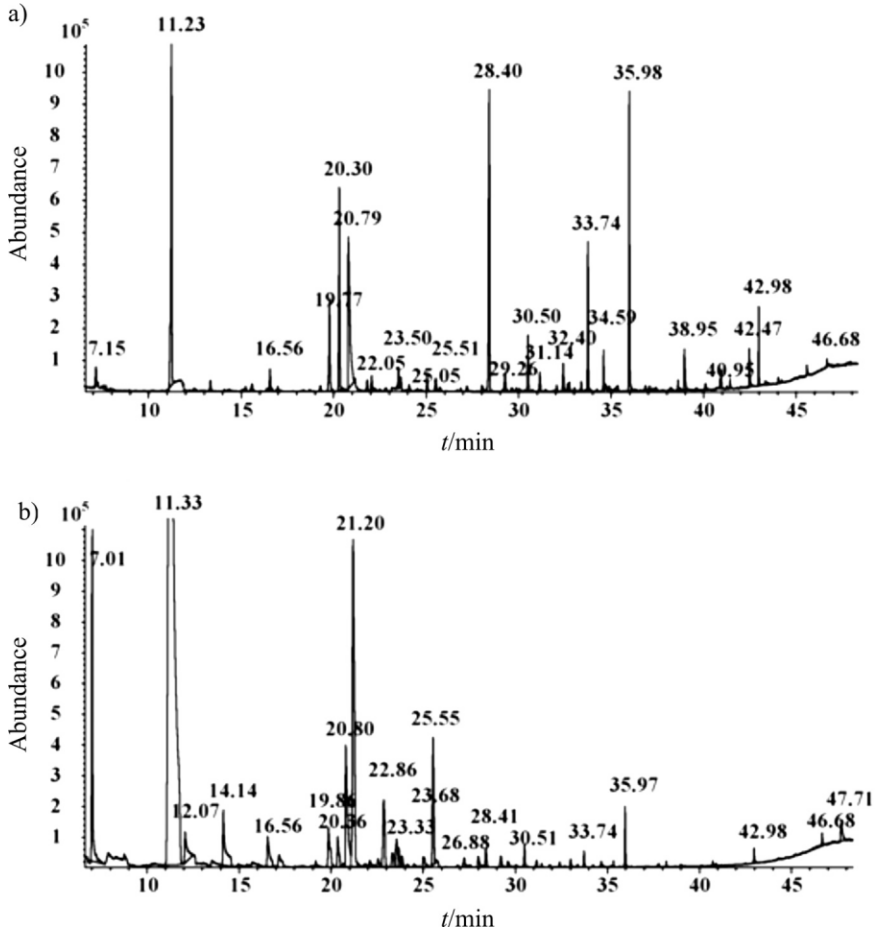

Fig. 4. Comparison of chromatograms obtained for tequila by HS-SPME-GC-MS using different fibers: a) orange fiber (Carbowax/divinylbenzene, CW/DVB), b) black fiber (Carboxen/polydimethylsiloxane, CAR/PDMS)

higher relative abundance with the black fiber than using the orange fiber, due to the polarity of the matrix. Thus, both fibers were used for a full characterization of volatile compounds in this study. The compounds identified in *Agave* alcoholic beverages are summarized in Table 2. There were 98 compounds detected using the HS-SPME-CG-MS technique. However, it is possible that other compounds are present at lower concentrations and they were not identified in this work. Almost all minor compounds reported previously for tequila, mezcal, sotol and bacanora (*6–8*) were detected here, but we detected new compounds such as azulene, cinnamol, butyrolactone, pyridine and others. The minor compounds detected include chemical groups such as aldehydes, ketones, alcohols, organic acids, fatty acid ethyl ester, furans, terpenes, napthalenes, and alkenes. Despite the low concentration of minor compounds, their presence is relevant since they harmonically synergize to produce the characteristic flavour and aroma for each type of beverage. Some minor compounds are produced during fermentation by microbial catabolism (*13,18*), whereas others derive from the raw material such as terpenes, napthalenes, hydrocarbons and long-chain fatty acids (*19*), thus they could be used as authenticity markers (*6*). There were 19 compounds detected in all beverages and 17 compounds were unique for each type of beverage as follows: 3-methyl-thio-1-propanol and nonanoic acid for pulque, 2-butylfuran for sisal, 3-methylcyclopentanone and benzofuran for mezcal from *A. durangensis*, 4-methoxybenzaldehyde for mezcal from *A. potatorum*, cinnamol for bacanora, 5-methyl-2-(1-methylethyl)-cyclohexanol for sotol, 1-methyl-4-(1-methylethenyl)-cyclohexene and geranyl ethyl ether for raicilla, heptanoic acid for mezcal from *A. salmiana*, naphtalene derivatives for mezcal from *A. angustifolia*, 2-methoxy--4-methyl-phenol, and 2-methyl-5-(1-methylethyl)-phenol for tequila. Nonanoic acid ethyl ester, 2-methyl--naphthalene and 2-acetylfuran had been proposed as authenticity markers for mezcal (from *A. angustifolia*), sotol and tequila, respectively (*20*), but these substances were also found in other alcoholic beverages analyzed here. This work updates the database of components that could be used as authenticity markers. The proposition of a compound as authenticity marker is not simple; fingerprinting methods are more reliable, as they are based on the presence and relative abundance of several compounds rather than just one of them.

The CA for the *Agave* alcoholic beverages based on the analysis of the presence or absence of minor compounds is shown in Fig. 5. The main cluster included distilled and non-distilled beverages obtained from the *Agave* plant, while sotol was not included. *Agave* and *Dasylirium* genera belong to the Agavaceae and Nolinaceae families, respectively. Although they are closely

Table 2. Compounds detected in *Agave* alcoholic beverages by HS-SPME-GC-MS

RT	Compound	Bac	Ang	Dur	Pot	Sal	Rai	Sis	Sot	Teq	Pul
7.02	2-Methyl-1-propanol*	1	1	1	1	1	1	1	1	1	1
7.89	3-Methyl-1-butanol acetate	1	1	1	1	0	1	1	0	1	1
7.97	3-Methyl-2-hexanol	0	0	0	0	0	0	0	1	0	1
8.34	Pentanoic acid ethyl ester	0	1	1	1	1	1	0	0	0	1
8.74	1-Butanol	0	1	1	1	1	1	1	1	0	1
9.76	Cyclopentanone	0	1	1	1	1	1	1	1	0	0
9.89	1-Methyl-4-(1-methylethenyl)-cyclohexene**	0	0	0	0	0	1	0	0	0	0
10.00	Pyridine	1	0	0	1	1	0	1	0	0	1
10.30	Limonene	0	1	0	0	1	1	1	0	0	1
10.75	Eucalyptol	0	0	0	1	0	1	0	0	0	0
10.89	3-Methylcyclopentanone**	0	0	1	0	0	0	0	0	0	0
10.92	3-Methylcyclopentanone	0	1	0	1	0	0	0	0	0	0
11.18	2-(3-)-Methyl-1-butanol*	1	1	1	1	1	1	1	1	1	1
11.19	1-Pentanol	1	0	0	1	1	1	0	1	0	0
11.27	3-Methyl-1-butanol formate	0	0	1	0	0	0	1	0	0	1
11.88	Hexanoic acid ethyl ester*	1	1	1	1	1	1	1	1	1	1
12.46	Cinnamol**	1	0	0	0	0	0	0	0	0	0
13.41	1,3,5-Trimethylbenzene*	1	1	1	1	1	1	1	1	1	1
13.91	3-Hydroxy-2-butanone	0	1	1	0	0	0	0	0	0	1
14.12	2-Butylfuran**	0	0	0	0	0	0	1	0	0	0
14.27	3-Hexen-1-ol	0	0	0	0	1	0	1	0	0	0
14.48	1-Methyl-4-propyl-benzene	0	1	1	1	1	1	1	1	0	1
15.23	1-Methyl-2-(1-methylethyl)-benzene*	1	1	1	1	1	1	1	1	1	1
16.08	Heptanoic acid ethyl ester	0	1	1	1	1	1	0	0	0	1
16.56	2-Hydroxypropanoic acid ethyl ester	0	1	1	1	1	1	1	1	1	1
16.69	1-Ethyl-2,4-dimethylbenzene	1	1	0	0	1	0	1	1	1	0
17.16	1-Hexanol	0	1	1	1	1	1	1	1	1	1
18.30	2-Nonanone	0	1	1	1	0	0	0	0	0	1
19.28	1,2,3,5-Tetramethylbenzene	0	0	1	0	0	0	1	1	1	0
19.37	1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene	1	0	0	0	1	0	0	0	0	0
19.86	1,3-Bis(1,1-dimethylethyl)-benzene*	1	1	1	1	1	1	1	1	1	1
20.29	Octanoic acid ethyl ester*	1	1	1	1	1	1	1	1	1	1
20.55	*p*-Menth-1-en-8-ol	0	1	1	0	0	0	0	0	0	1
20.65	5-Ethenyl-tetrahydro-5-trimethyl-2-furanmethanol	1	1	1	0	0	1	1	0	0	0
20.66	Acetic acid*	1	1	1	1	1	1	1	1	1	1
21.14	Furfural*	1	1	1	1	1	1	1	1	1	1
21.20	3-Furaldehyde	0	1	0	0	0	0	0	0	1	0
21.37	1-Heptanol	0	0	0	1	0	1	0	0	0	1
21.87	1,2,3,4-Tetramethylbenzene*	1	1	1	1	1	1	1	1	1	1
22.59	Benzofuran**	0	0	1	0	0	0	0	0	0	0
22.86	1-(2-Furanyl)ethanone*	1	1	1	1	1	1	1	1	1	1
23.34	Benzaldehyde*	1	1	1	1	1	1	1	1	1	1
23.42	Geranyl ethyl ether**	0	0	0	0	0	1	0	0	0	0
23.52	3-Methyl-6-(1-methylethylidene)-cyclohexene*	1	1	1	1	1	1	1	1	1	1
24.07	5-(Dimethylamino)-benzofurazan	0	0	1	0	0	1	0	0	1	0
24.41	Nonanoic acid ethyl ester	0	1	1	0	0	0	0	0	0	1
24.50	Propanoic acid	0	1	1	1	1	1	0	0	0	0
24.78	2,3-Butanediol	0	0	0	0	0	1	0	0	0	1
25.04	3,7-Dimethyl-1,6-octadien-3-ol	1	1	1	1	1	1	1	0	0	1
25.50	5-Methyl-2-furancarboxaldehyde*	1	1	1	1	1	1	1	1	1	1
25.65	2-Methylpropanoic acid	0	1	1	1	1	0	0	0	1	0

Table 2. continued

RT	Compound	Bac	Ang	Dur	Pot	Sal	Rai	Sis	Sot	Teq	Pul
26.06	2-Methylbenzofuran	1	1	1	0	0	1	1	0	0	0
26.68	Decanoic acid methyl ester	0	1	0	0	0	0	1	0	0	0
26.85	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	1	1	1	1	1	1	1	0	1	1
27.14	2-Acetylfuran	0	1	1	0	0	1	0	1	1	0
27.41	Butyrolactone	0	0	0	0	0	0	0	1	0	1
27.54	2-Furancarboxylic acid ethyl ester	1	1	0	1	0	1	1	1	0	1
27.93	Butanoic acid	1	1	1	1	1	1	0	1	1	1
28.38	Decanoic acid ethyl ester*	1	1	1	1	1	1	1	1	1	1
28.44	5-Methyl-2-(1-methylethyl)-cyclohexanol**	0	0	0	0	0	0	0	1	0	0
29.14	*p*-Allyl-anisole	0	1	0	1	0	1	0	0	0	1
29.20	2-Furanmethanol	1	0	1	0	1	0	1	0	0	0
29.52	3-Methylbutanoic acid	0	1	1	1	0	0	0	0	0	0
29.63	3-Methylpentanoic acid	0	0	0	0	1	0	1	0	0	0
29.87	Butanedioic acid diethyl ester	0	1	0	0	0	0	0	0	1	1
30.50	4-Trimethyl-3-cyclohexene-1-methanol*	1	1	1	1	1	1	1	1	1	1
31.09	3-(Methylthio)-1-propanol**	0	0	0	0	0	0	0	0	0	1
31.13	Naphthalene	1	1	1	1	1	1	1	1	1	0
31.14	Azulene	0	0	0	0	1	0	0	0	1	1
31.86	Pentanoic acid	0	1	1	1	1	1	0	0	0	0
31.95	1,2,3,5,6,8-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene**	0	1	0	0	0	0	0	0	0	0
32.04	1-Methoxy-4-(1-propenyl)-benzene	0	0	0	1	0	1	0	0	0	1
32.41	Methylsalicylate	1	1	0	1	1	0	1	1	1	0
32.73	3,7-Dimethyl-6-octen-1-ol	0	1	0	0	1	1	1	0	1	1
33.38	2,3-Dihydro-1-H-inden-1-ol	1	0	0	1	0	0	0	0	0	0
33.38	3,5-Dimethylbenzaldehyde	0	0	1	0	1	1	1	1	1	1
33.74	Acetic acid 2-phenylethyl ester	1	1	1	1	1	1	1	0	1	1
33.83	1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	0	0	0	0	1	0	0	1	0	0
33.96	1,2,3,4-Tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene**	0	1	0	0	0	0	0	0	0	0
34.22	2-Methylnaphthalene	1	1	1	0	1	1	0	1	0	0
34.57	Dodecanoic acid ethyl ester	1	1	1	0	0	1	1	1	1	0
34.61	Hexanoic acid	1	1	1	1	1	0	0	1	1	1
34.75	2-Methoxyphenol	1	0	1	0	0	0	1	0	0	0
35.20	Benzyl alcohol	1	1	0	1	1	0	1	0	0	0
35.35	Benzenepropanoic acid ethyl ester	0	1	1	0	0	0	0	0	0	0
35.96	Phenylethyl alcohol*	1	1	1	1	1	1	1	1	1	1
36.37	1-Ethylnaphthalene**	0	1	0	0	0	0	0	0	0	0
36.85	2-Methoxy-4-methylphenol**	0	0	0	0	0	0	0	0	1	0
36.98	Heptanoic acid**	0	0	0	0	1	0	0	0	0	0
37.44	2,6-Dimethylnaphthalene	0	1	1	0	0	1	0	0	1	0
37.78	Phenol*	1	1	1	1	1	1	1	1	1	1
38.11	4-Methoxybenzaldehyde**	0	0	0	1	0	0	0	0	0	0
38.75	Octanoic acid	1	1	1	1	1	1	1	0	1	1
40.76	Nonanoic acid**	0	0	0	0	0	0	0	0	0	1
41.35	2-Methyl-5-(1-methylethyl)-phenol**	0	0	0	0	0	0	0	0	1	0
41.45	1,6-Dimethyl-4-(1-methylethyl)-naphthalene**	0	1	0	0	0	0	0	0	0	0
42.01	Dibenzofuran	0	1	1	0	1	0	0	0	0	0
42.45	Decanoic acid	0	1	1	0	1	1	1	0	1	1
42.97	2,4-Bis(1,1-dimethylethyl)-phenol*	1	1	1	1	1	1	1	1	1	1

RT=retention time in the DB-WAX column; 1=presence, 0=absence; *compounds found in all beverages and **compounds unique for each beverage; Bac=bacanora, Ang=mezcal from *A. angustifolia*, Dur=mezcal from *A. durangensis*, Pot=mezcal from *A. potatorum*, Sal=mezcal from *A. salmiana*, Rai=raicilla, Sis=sisal, Sot=sotol, Teq=tequila and Pul=pulque

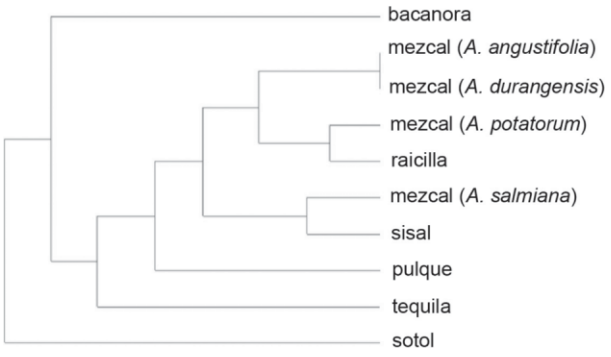

Fig. 5. Dendrogram of cluster analysis for the minor compounds in the ethnic *Agave* alcoholic beverages using the UPGMA method with Jaccard coefficient

related phylogenetically, they have significant structural and biochemical differences and therefore their secondary metabolites differ (*4*).

Conclusions

We detected 105 compounds in the *Agave* alcoholic beverages analyzed here, 11 of them were classified as major compounds and the rest were classified as minor compounds. Seventeen minor compounds were unique for each type of beverage and they could be used as authenticity markers. The minor compounds group includes alcohol, ketones, esters, organic acids, furans, terpenes, alkenes and others. According to this study, most of the compounds found in the *Agave* alcoholic beverages are similar to those reported for whisky, cachaça and other alcoholic beverages. However, compounds such as azulene, cinnamol, and others that could be used as authenticity markers were found. Cluster analysis (CA) showed that *Agave* alcoholic beverages could be distinguished by multivariate analysis of major compounds, while the analysis of minor compounds provides better fingerprinting.

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