

Major and Minor Compounds in A Mexican Spirit, Young Mezcal Coming from Two *Agave* Species

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Abstract

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Major and minor compounds in a traditional Mexican spirit, young mezcal from *Agave angustifolia* Haw. and *Agave potatorum* Zucc., were characterised using gas chromatography and solid phase microextraction-gas chromatography-mass spectrometry. A large variability in both mezcal samples was detected in the content of methanol, higher alcohols, acetic acid, and ethyl acetate. However, their values were below the maximum concentration permitted by the Mexican Standards. The minor compounds identified by mass spectrometry included alcohols, esters, ketones, acids, and furanes. The similarities found between mezcal from *Agave angustifolia* and *Agave potatorum* may be due to their processing methods. In addition, mezcals contain unique compounds that can be used as markers to identify the products of different origins.

Keywords: mezcal; volatile compounds; *Agave angustifolia* Haw.; *Agave potatorum* Zucc.; GC-FID; SPME-GC-MS

In Mexico, different *Agave* plants are exploited for the production of alcoholic distilled beverages which are generically known by the name mezcal, for example, *Agave tequilana* Weber blue variety is used for the preparation of tequila; *Dasyrilon* spp. and *Agave angustifolia* Haw. for the production of sotol and bacanora, respectively; while *A. salmiana*, *A. angustifolia* Haw., and *A. potatorum* Zucc. are used for the production of mezcal (LAPPE-OLIVERASO *et al.* 2008). Mezcal is produced in different regions of Mexico with the denomination of origin,

which includes the states of Oaxaca, Durango, Guerrero, San Luis Potosí, Zacatecas, Guanajuato, and Tamaulipas. Traditionally, in Oaxaca, mezcal is obtained by fermentation and distillation of sugars from *Agave* plants as *Agave angustifolia* Haw. and *Agave potatorum* Zucc. In addition, different kinds of mezcal are made by traditional producers in Oaxaca: Mezcal type I, obtained exclusively from *Agave* sugars, and Mezcal type II, produced using 80% of *Agave* sugars and 20% of sugar from other sources (NOM-070-SCFI-1994). In each

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category, there are three types, young (blanco) mezcal without maturation, rested (reposado) mezcal, which is matured for at least 2 months in oak barrels, and aged (añejo) mezcal, which is matured for up to 12 months. Sometimes, young and aged mezcals are conditioned with larvae of Agave worms and are known as worm's beverage. Mezcal from Oaxaca has reached national and international recognition in the last few years, but at the moment the studies on the chemical compositions of different varieties are few. Several studies on the composition of volatiles have been performed on tequila and mezcal from *A. salmiana*, while there is little information on mezcal from *A. angustifolia* and *A. potatorum* (LÓPEZ & GUEVARA 2001; DE LEÓN-RODRÍGUEZ *et al.* 2006; LACHENMEIER *et al.* 2006). In addition, in most of these studies the geographic location, processing conditions, and aging time are not known. Therefore, in the present work, samples of young mezcal from *Agave angustifolia* Haw. and *Agave potatorum* Zucc. produced by the traditional method (spontaneous fermentation) were collected from three regions in Oaxaca, and their major and minor compounds were characterised using gas chromatography (GC) and solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS).

MATERIAL AND METHODS

Material. The analyses were carried out on eleven samples of young mezcals done by traditional producers at different locations in Oaxaca, Mexico. Eight mezcals from *Agave angustifolia* Haw. were collected in the region of Matatlán and Tlacolula, Oaxaca, while three brands from *Agave potatorum* Zucc. were obtained from Sola de Vega, Oaxaca.

Analysis of major compounds by GC-flame ionisation detection. Quantitative analysis of the major compounds, ethanol, methanol, 1-butanol, 2-butanol, propanol, 2-methyl-propanol, and 3-methyl-butanol was based on the Mexican standards (NOM-070-SCFI-1994) for the analysis of mezcal. A Perkin Elmer (Walnut Creek, USA) gas chromatograph fitted with a flame ionisation detector and a 30 m × 0.25 mm i.d., 0.25 µm film thickness, HP-INNOWAX fused silica capillary column was used. The injector and detector were kept at 180°C and 230°C, respectively. Helium at a

flow rate of 3 ml/min was used as the carrier gas. The temperature program was the following: 40°C for 3 min, then raised at 3°C/min up to 120°C and at 6°C/min to 200°C, which was held for 20 minutes. Standard curves for the quantification of the major compounds were constructed with a 40% (v/v) ethanol solution at 20°C. Sec-butyl acetate was used as internal standard.

Analysis of minor compounds by SPME-GC-MS. Two milliliters of each mezcal sample were equilibrated in a sealed vial at room temperature for 2 h (LÓPEZ & GUEVARA 2001). After this time, volatile compounds were extracted with a SPME fiber, carbowax/divynilbenzene (CAR/DVB) (Supelco, Bellefonte, USA). After this extraction procedure, a manual SPME holder containing fiber was introduced in the vial and was exposed to the sample headspace for 30 min at room temperature. Desorption of volatiles was conducted in a GC injector with a SPME inlet liner (0.75 mm i.d., Supelco) for 1 min at 200°C. An HP model 5890 gas chromatograph equipped with a HP-FFAP (30 m × 0.32 mm i.d., film thickness × 0.25 µm) fused silica capillary column was used. The injector and detector temperatures were 180°C and 230°C, respectively. As the carrier gas, helium was used at a flow rate of 1 ml/minutes. The oven temperature was programmed from 40°C for 3 min with a ramp of 3°C/min to 120°C and this temperature was held for 5 minutes. The GC was interfaced with an HP 5972 MS for the detection and quantification of the volatile compounds. The transfer line was 250°C. Electron impact mass spectra were scanned at 70 eV in the *m/z* range 50–500 mass units. The volatile compounds were identified by comparing their mass spectra with the computerised spectral databases.

Statistical analysis. The data were analysed by one-way analysis of variance (ANOVA using the Origin V7.5 software (Originlab, Northampton, USA). Statistical significance was assumed at below the 0.05 probability level. Box and whisker plots were used for the data visualisation.

RESULTS AND DISCUSSION

Major compounds

The concentrations of major compounds in mezcal samples are summarised in Figure 1, where a large variability in the concentrations of the major

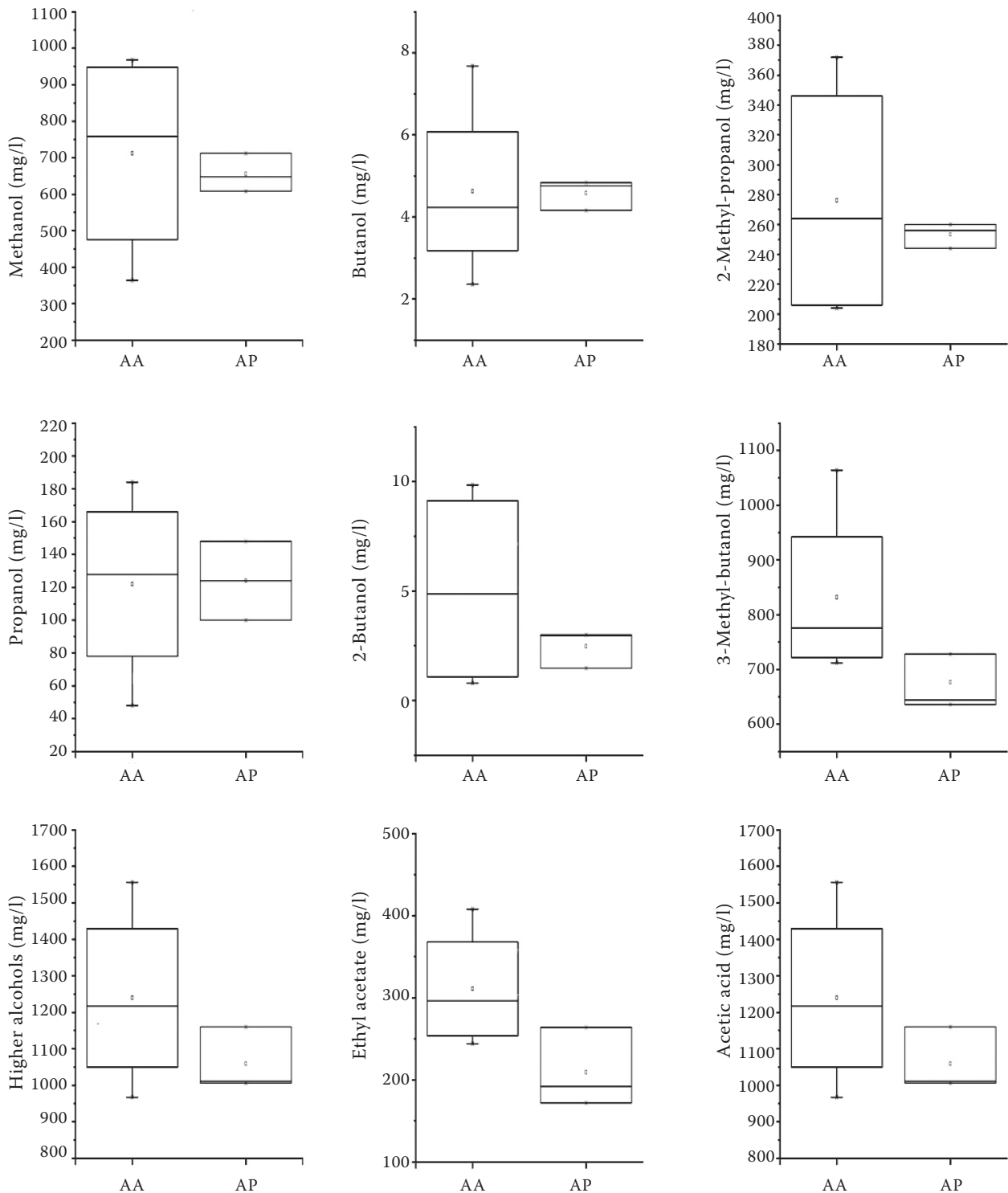


Figure 1. Box chart of the methanol, butanol, 2-methyl-propanol, propanol, 2-butanol, 3-methyl-butanol, higher alcohols, ethyl acetate, and acetic acid contents of mezcal from *Agave angustifolia* Haw. (AA) and *Agave potatorum* Zucc. (AP)

compounds can be observed. High variability in the contents of methanol, higher alcohols, acetic acid, and ethyl acetate has been reported for the Mexican spirits obtained with rudimentary

production methods, such as sotol and bacanora (LACHENMEIER *et al.* 2006). However, it can be observed that methanol concentrations in the mezcal samples were below the maximum limit permitted

by the Mexican legislation, 1200 mg/l anhydrous alcohol (NOM-070-SFCI-1994). In addition, the ANOVA test showed no significant differences in the concentration of methanol between the mezcal from *Agave angustifolia* and that from *Agave potatorum* ($P < 0.05$). Other studies on Mexican Agave spirits such as tequila, sotol, bacanora, and mezcal from *A. salmiana* showed that this alcohol was within the limits established by the Mexican law (DE LEÓN-RODRÍGUEZ *et al.* 2006; LACHENMEIER *et al.* 2006). Methanol may be produced during some steps of mezcal processing like cooking. During the Agave pine cooking, methanol is directly formed from pectin and lignin, which are present in the cell wall. It is an undesirable component in the final product due to its high toxicity for humans (LACHENMEIER *et al.* 2006).

On the other hand, mezcal produced from *Agave potatorum* showed the lowest amounts of butanol, 1-propanol, 1-propanol, 2-methyl, 2-butanol, and 1-butanol, 3-methyl and, consequently, of higher alcohols (Figure 1). Furthermore, mezcal samples showed significant differences in the concentration of higher alcohols ($P < 0.05$) without exceeding the limits established by the Mexican standards, i.e. 400–1600 mg/l of anhydrous ethanol, in both mezcal samples (NOM-070-SCFI-1994). An excessive concentration of higher alcohols can result in a strong pungent and 'fusel-like' smell and taste, whereas optimal levels impart a fruity character (CHRISTOPH & BAUER-CHRISTOPH 2007).

Mezcal from *A. potatorum* also presented lower values of ethyl acetate and acetic acid than mezcal from *A. angustifolia*, these differences being statistically significant ($P < 0.05$). The high concentrations of acetic acid and ethyl acetate in mezcal from *A. angustifolia* could be due to a major contamination of the fermentative musts with acetic acid bacteria or a poor distillation technique, and may cause an off-flavour at certain levels. In addition, ethyl acetate, mainly produced as a result of acetic acid esterification, is the main ester which occurs in fermented products and the distillates from them; it contributes significantly to a solvent-like nail polish off-flavour at high levels.

Minor compounds

A total of 24 compounds were detected by GC-MS in mezcal samples (Table 1). It can be seen that most of them were similar to those reported for

tequila and other alcoholic beverages. Table 1 lists the similarities between the volatile profiles of mezcals from *A. angustifolia* and *A. potatorum*, among the compounds detected were alcohols, esters, acids, furans, naphthalenes, and phenols. Ethanol, propanol, 1-propanol, 2-methyl, 1-butanol, 1-butanol, 3-methyl and phenylethyl alcohol were common to all samples. Alcohols such as 1-butanol, 3-methyl and phenylethyl alcohol are responsible for the sweet notes of most alcoholic beverages (CHRISTOPH & BAUER-CHRISTOPH 2007).

Among esters, ethyl acetate, 1-butanol, 3-methyl acetate, octanoic acid ethyl ester, and decanoic acid ethyl ester were detected in all the samples, while dodecanoic acid ethyl ester was only found in *A. angustifolia*. It is well known that low-boiling ethyl esters like octanoic acid ethyl ester, as well as acetates like ethyl acetate, are of great importance to the flavour of distilled spirits (CHRISTOPH & BAUER-CHRISTOPH 2007).

Acetic acid, propanoic acid 2-hydroxy- and 3-methyl butanoic acid were found in all samples, while acetic acid 2-phenyl ethyl ester was only detected in mezcal from *A. potatorum*. Butanoic acid was only found in *A. angustifolia*. On the other hand, Maillard compounds like furfural and 5-methyl-2-furancarboxaldehyde, were detected in both mezcals, while 2-furanmethanol was only detected in mezcal from *A. potatorum*. The presence of toxic compounds like furfural could be explained by the overcooking of Agave. It has been reported that during tequila production Maillard products are generated mainly during Agave cooking (Table 1), having an impact on the characteristic flavour of this beverage (MANCILLA-MARGALI & LÓPEZ 2002).

On the other hand, phenol and naphthalene were detected in all mezcals. It seems likely that some of the phenols are formed during the cooking and/or distillation steps of mezcal production. Furthermore, naphthalene has been detected in mezcal from *A. salmiana*, sotol, and bacanora (Table 1) (LÓPEZ & GUEVARA 2001; DE LEÓN-RODRÍGUEZ *et al.* 2006).

According to the data obtained in this study, it can be seen that butanoic acid, 2-furanmethanol, acetic acid, 2-phenyl ethyl ester and dodecanoic acid, ethyl ester might be used as markers in young mezcals from *A. angustifolia* and *A. potatorum*. Previous studies suggested that nonanoic acid ethyl ester and 4-methoxybenzaldehyde could be

Table 1. Volatile compounds identified in mezcal from *Agave angustifolia* Haw. and *Agave potatorum* Zucc. by SPME-GC-MS

RT	Volatile compound	Mezcal		Literatura data ^a		
		AA	AP	AS	AT	AD
1.5	ethyl acetate	+	+		+	+
2.1	ethanol	+	+	+	+	+
4.3	propanol	+	+	+	+	+
6.1	1-propanol, 2-methyl	+	+	+		+
6.4	1-butanol, 3-methyl-, acetate	+	+	+	+	+
10.1	1-butanol, 3-methyl	+	+	+	+	+
13.4	propanoic acid, 2-hydroxy-	+	+			
15.8	propanoic acid, 2-hydroxy-ethyl ester	+	+			
19.6	octanoic acid, ethyl ester	+	+	+	+	+
20.5	acetic acid	+	+	+	+	+
21.3	furfural	+	+	+	+	+
22.9	ethanone, 1-(2-furanyl)	+	+		+	+
24.2	propanoic acid	+	+	+	+	+
25.3	propanoic acid, 2 methyl-	+	+		+	+
25.7	2-furancarboxaldehyde, 5-methyl-	+	+			
27.8	butanoic acid	+		+		+
28.0	decanoic acid, ethyl ester	+	+	+	+	+
29.4	butanoic acid, 3-methyl	+	+		+	+
29.6	2-furanmethanol		+			+
31.9	naphthalene	+	+	+		+
34.6	acetic acid, 2-phenyl ethyl ester		+		+	+
35.2	dodecanoic acid, ethyl ester	+			+	+
37.1	phenylethyl alcohol	+	+	+	+	+
39.2	phenol	+	+			+

RT = retention time, AA = *Agave angustifolia* Haw., AP = *Agave potatorum* Zucc., AS = *Agave salmiana*, AT = *Agave tequilana* Weber, AD = *Dasyrilon* spp.

^aData reported on several types of mezcal (LÓPEZ & GUEVARA 2001; DE LEÓN-RODRÍGUEZ *et al.* 2006)

used as markers, but these compounds were not found in the samples analysed here. This could be attributed to the processing conditions and ageing time of the different mezcal samples used in the different studies. It is well known that ageing of distilled spirits is an important technological step, where different components of a fresh distillate may react during the maturation period, and the concentrations of compounds like nonanoic acid ethyl ester can be increased (VALLEJO-CÓRDOBA

et al. 2004). Future work on the technological aspects like distillation and ageing of mezcal should be considered.

CONCLUSIONS

The quantitative variation of major compounds in young mezcal from *A. angustifolia* Haw. and *A. potatorum* Zucc. may be due to the processing

methods. However, the quality of these Mexican alcoholic beverages complies with the Mexican Standards. On the other hand, similarities found between minor compounds may be due to the raw material (*Agave* species) and conditions of mezcal processing. In the same way, the mezcals tested contain compounds such as 2-furanmethanol and dodecanoic acid, ethyl ester, which might be used as markers to discriminate from each other.

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