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# Volatile Compound Profiles in Mezcal Spirits as Influenced by Agave Species and Production Processes

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**Abstract:** Mezcal is a traditional Mexican spirit produced by distilling fermented *Agave*. The effects of *Agave* species, origin, and season on the volatile compound profile were studied in mezcal from Oaxaca, Mexico. Liquid-liquid extraction was used to isolate volatile compounds from mezcals made from *Agave angustifolia* Haw. and *Agave potatorum* Zucc. These compounds were identified using gas chromatography-mass spectrometry (GC-MS). Eighty-four volatile compounds were identified, including alcohols, esters, fatty acids, ketones, furans, and others. Using variance analysis, it was possible to observe significant differences for the 26, 24, and 10 compounds in mezcal samples that differed based on *Agave* species, origin, and season. 3-Ethyl-phenol was identified only in samples of mezcal from *A. angustifolia*, and this volatile compound could be used as an authentic marker of mezcal from *A. angustifolia* ($p \leq 0.01$).

**Keywords:** alcoholic beverages; gas chromatography-mass spectrometry; volatile compounds; mezcal

## 1. Introduction

Mexico is the point of origin and center of biodiversity of the *Agave* genus, which plays an important role in the country's culture as a result of its multiple uses, especially in terms of food, fibers, and spirits [1]. Alcoholic beverages from the *Agave* species are very popular in Mexico and worldwide. For example, *Agave tequilana* is used in the production of tequila, *A. angustifolia* is used to produce bacanora, and *A. salmiana*, *A. cupreata*, *A. duranguensis*, *A. fourcroydes*, *A. angustifolia*, and *A. potatorum* are used in the production of mezcal [2–4]. Mezcal is an artisan distilled alcoholic beverage produced in different regions of Mexico with a designation of origin [5]. Oaxaca, Mexico is one region where mezcal is produced from *A. angustifolia* and *A. potatorum*. The artisan production process involves four stages: cooking the agave stem (leafless flowering axis), mashing the cooked stem, fermenting the mashed stem, and distilling the fermented stem [4,6].

Among the factors that have an influence on the artisanal process of making mezcal are *Agave* species and origin [2,4,7], initial sugar concentration, environmental conditions, native yeast strain, and, in certain cases, the addition of ammonium sulfate or ammonium chloride to the fermentation must [4,6,8–10]. Another important parameter that ultimately defines the quality of agave beverages is the distillation system used [11,12]. Studies by Vera-Guzmán et al. [4] found that major volatile compounds in mezcal differed between *Agave* species and fermentation conditions. Furthermore, Vera-Guzmán et al. [6], through the use of traditional and spontaneous mezcal fermentation processes, have shown that chemical composition varied significantly among artisanal distilleries due to the specific characteristics of the core of the agave plants, the musts used, and the natural yeasts.

The quality and authenticity of mezcal is a highly relevant issue because of the beverage's unique alcoholic flavor, which is the result of the volatile and non-volatile compounds, the direct precursors of which come from the raw *Agave* itself. These include fatty acids, ranging from capric to lignoceric, free fatty acids, β-sitosterol, and groups of mono-, di-, and triacylglycerols [13,14], as well as fructans, the principal carbohydrate of the *Agave* [15]. Fructans could form Maillard compounds, such as furans, pyrans, and ketones, as a result of higher temperatures and a lower pH in the agave cooking process [16]. On the other hand, the fermentation process generates ethanol, higher alcohols, esters, organic acid and others [4,6,8–10]. Some of these volatile compound are of greater importance than others due to their concentrations or aromatic characteristics [12,17], and some could be specific to the *Agave* species.

In Oaxaca, there are many mezcal distilleries that use artisan processes, and these characteristics generate specific spirits that are appreciated by the consumers because of their sensory properties. Several factors have an influence on the flavor of mezcal; however, few studies have been performed regarding the impact *Agave* species, place of origin, and production season have on this process. The aim of this study is to evaluate the effect of agave species, origin, and season on the minor volatile compound profiles in mezcals from *A. angustifolia* and *A. potatorum* in Oaxaca.

## 2. Materials and Methods

### 2.1. Mezcal Samples

The agave stem used in this study was harvested from *Agave angustifolia* Haw. plantations located in the communities of Matatlan (16°52′30′′ N, 96°23′44′′ W, 1740 MASL) and Tlacolula (16°58′42′′ N, 96°32′11′′ W, 1660 MASL), and from *A. potatorum* plantations located in Sola de Vega (16°30′13′′ N, 97°59′03′′ O, 1440 MASL) in Oaxaca, Mexico. The aforementioned communities have a temperate, sub-humid climate with summer rainfall. The points of origin were taken as being the artisan distilleries where the mezcal is made, as origin is one variable studied in this paper. In the distilleries at Tlacolula and Matatlan, the process for making mezcal with *A. angustifolia* begins with cooking the agave stems in rustic ovens (earth ovens) for 72 h. The cooked agave stem was then mashed using a horse-driven stone mill, and bagasse juice was transferred to wooden tanks. Water is then added to start the fermentation process. In both locations, the fermentation is carried out without the addition of yeast inoculum, using only native microflora found in the environment, which is why it is considered to be a spontaneous process. After fermentation, the bagasse and fermented juice go into a wood-fired copper pot still, and the first distillation process is completed. Mezcal goes through two further distillations, creating a product with a 45% alcohol content ($v/v$). To evaluate the impact of production seasons on mezcal made with *A. angustifolia*, two samples per season were obtained for spring, fall, and winter from Tlacolula, while two samples per season for spring and fall were obtained in Matatlan.

Six samples of *A. potatorum* mezcal from the spring were obtained from the community of Sola de Vega. Firstly, the *A. potatorum* stems were cooked for three days in rustic ovens (earth ovens). Then, the cooked agave stem was mashed by hand using large wooden mallets and then placed in big wooden tubs known as "canoes", where they are left to ferment using native microflora as a spontaneous process. The fermented stem was then distilled twice by heating it in earthenware pots and condensing and collecting the resulting steam. Finally, a product with an alcohol content of 45% ($v/v$) was obtained. To summarize, six samples from Tlacolula (two per season) and four samples from Matatlan (two per season) were obtained for mezcal *A. angustifolia*, while six samples of *A. potatorum* mezcal was sampled in Sola de Vega from the spring season. All samples were stored at 8 °C in the lab until their analysis using GC-MS.

*2.2. Extraction of Volatile Compounds*

Fifteen milliliters of mezcal were extracted with 15 mL of dichloromethane of ≥99.9% purity (Reg. 650463, Sigma, St. Louis, MO, USA). The extract was dried using anhydrous sodium sulfate of ≥99.0% purity (Reg. 239313, Sigma, St. Louis, MO, USA) and then concentrated using Kuderna-Danish apparatus to a volume of 250 µL. Each sample was extracted in duplicate and then analyzed using GC-MS [18].

*2.3. Analysis of Aromatic Compounds Using GC-MS*

Each sample was injected into a Hewlett-Packard 5890 Series II gas chromatographer linked to a Hewlett-Packard 5972 series mass selective detector (Hewlett Packard, Bothell, WA, USA). An HP-FFAP column (30 m, 0.25 mm ID, 0.32 µm thickness, San Fernando, CA, USA) was used for chromatographic separation. The temperature of the injector was maintained at 180 °C throughout the analysis. Helium at 1 mL/min was used as the carrier gas. GC conditions were as follows: 40 °C for 3 min, which was increased by 6 °C/min to 120 °C and then maintained for 1 min. The temperature was then increased to 200 °C at a rate of 3 °C/min. The ion source temperature was set at 230 °C. The mass spectra in the electron impact (EI) mode were generated at 70 eV. The Kovats index of the volatile compounds was calculated using a fatty acid series (C2–C18) present in the sample. The compounds were identified by comparing their mass spectra to those obtained in the NIST98 library of the MS database with a ≥80% match. Some specific compounds were confirmed using standards (2-methyl-1-propanol, 309435; 3-methyl-1-butanol, 294829; acetic acid, 695092; fatty acids, EC10A, with ≥99 purity, Sigma, St. Louis, MO, USA) or published literature [16,19]. Quantitative data was obtained using 3-octanol (≥98.5% purity; Reg. 93856, Sigma, St. Louis, MO, USA) as an internal standard. All tests were performed twice.

*2.4. Statistical Analysis*

Variance analysis per volatile compound using a lineal model of a completely random design was carried out by disaggregating the source treatments into three variables to be studied: communities of origin, agave species, and sampling seasons, which were used to test the significant differences ($p \leq 0.01$) for each disaggregated source in relation to the volatile compounds in the mezcal samples analyzed. The sources of variation were Agave species, sample origin (Tlacolula, Matatlan, and Sola de Vega), nested in species, and sampling season (spring, fall and winter), nested in communities of origin. All data analysis was carried out using SAS software (Statistical Analysis System Institute, 9th ed. Cary, NC, USA, 2002).

## 3. Results and Discussion

*3.1. Volatile Compounds in Mezcal from A. angustifolia and A. potatorum*

A total of eighty-four and eighty-one aroma compounds were isolated using liquid-liquid extraction and identified by GC-MS in mezcal produced from *A. angustifolia* and *A. potatorum*, respectively (Tables 1 and 2). The main volatile compounds were alcohols, esters and organic acids, as well as some phenols, ketones, furans, naphthalene and others. It is noteworthy that both esters and alcohols are also the main compounds found in other Mexican distilled spirits, such as tequila and mezcal from *Agave salmiana* and *A. duranguensis* [2,18–20].

**Table 1.** Mean squares of the ANOVA's of minor volatile compounds evaluated of mezcal by *Agave* species, origin, and season.

| Compound Number | Compound Name | Agave Species | Origins | Seasons |
|---|---|---|---|---|
| | **Alcohols** | | | |
| 1 | 2-Methyl-1-propanol | 6.98 $^{ns}$ | 0.85 $^{ns}$ | 1.16 $^{ns}$ |
| 2 | 1-Butanol | 0.05 $^{ns}$ | 0.41 $^{ns}$ | 0.02 $^{ns}$ |
| 3 | 3-Methyl-1-butanol | 49.95 $^{ns}$ | 3.35 $^{ns}$ | 13.50 $^{ns}$ |
| 4 | 3-Methyl-3-buten-1-ol | 0.26 $^{ns}$ | 0.15 $^{ns}$ | 0.15 $^{ns}$ |
| 5 | Cyclopentanol | 0.08 $^{ns}$ | 1.23 $^{**}$ | 0.40 $^{*}$ |
| 6 | 4-Methyl-1-pentanol | 0.27 $^{**}$ | 0.02 $^{ns}$ | 0.02 $^{ns}$ |
| 7 | 3-Methyl-1-pentanol | 0.45 $^{**}$ | 0.01 $^{ns}$ | 0.02 $^{ns}$ |
| 8 | 3-Methyl-cyclopentanol | 2.22 $^{*}$ | 19.29 $^{**}$ | 3.70 $^{**}$ |
| 9 | 1-Hexanol | 0.36 $^{ns}$ | 3.47 $^{*}$ | 1.77 $^{ns}$ |
| 10 | 3-Ethoxy-1-propanol | 61.95 $^{**}$ | 4.61 $^{ns}$ | 0.05 $^{ns}$ |
| 11 | 2,3-Butanediol | 1.29 $^{ns}$ | 2.86 $^{**}$ | 2.82 $^{**}$ |
| 12 | 1-Octanol | 0.01 $^{ns}$ | 0.10 $^{ns}$ | 0.04 $^{ns}$ |
| 13 | Decanol | 0.04 $^{ns}$ | 2.43 $^{**}$ | 0.14 $^{ns}$ |
| 14 | Benzyl alcohol | 3.22 $^{ns}$ | 5.22 $^{*}$ | 3.21 $^{ns}$ |
| 15 | Phenylethyl alcohol | 1471.63 $^{ns}$ | 15.91 $^{ns}$ | 661.05 $^{ns}$ |
| 16 | Benzene propanol | | | 0.04 $^{ns}$ |
| 17 | 1-Hexadecanol | 0.05 $^{ns}$ | 0.81 $^{ns}$ | 0.09 $^{ns}$ |
| | **Esters** | | | |
| 18 | 3-Methyl-1-butanol acetate | 0.76 $^{ns}$ | 0.05 $^{ns}$ | 0.09 $^{ns}$ |
| 19 | Hexanoic acid ethyl ester | 0.08 $^{ns}$ | 1.14 $^{**}$ | 0.09 $^{ns}$ |
| 20 | 2-Hidroxy propanoic acid ethyl ester | 10.92 $^{ns}$ | 56.44 $^{**}$ | 3.58 $^{ns}$ |
| 21 | Octanoic acid ethyl ester | 1.88 $^{ns}$ | 11.42 $^{**}$ | 0.56 $^{ns}$ |
| 22 | Decanoic acid ethyl ester | 0.90 $^{ns}$ | 26.00 $^{**}$ | 0.56 $^{ns}$ |
| 23 | Butanedioic acid diethyl ester | 0.61 $^{ns}$ | 0.05 $^{ns}$ | 2.16 $^{*}$ |
| 24 | Acetic acid 2 phenylethyl ester | 137.33 $^{ns}$ | 42.25 $^{ns}$ | 57.04 $^{ns}$ |
| 25 | Dodecanoic acid ethyl ester | 37.19 $^{**}$ | 486.95 $^{**}$ | 71.68 $^{**}$ |
| 26 | 2-Cyclohexene-1-acetic acid, 1-hydroxy ethyl ester | 1.79 $^{ns}$ | 0.003 $^{ns}$ | 0.35 $^{ns}$ |
| 27 | Tetradecanoic acid ethyl ester | 15.12 $^{ns}$ | 33.94 $^{*}$ | 8.54 $^{ns}$ |
| 28 | Hexadecanoic acid ethyl ester | 110.82 $^{**}$ | 162.00 $^{**}$ | 187.8 $^{**}$ |
| 29 | (R) 2-Butenedioic acid diethyl ester | 0.46 $^{ns}$ | <0.01 $^{ns}$ | 0.30 $^{ns}$ |
| 30 | 9,12-octadecadienoic acid ethyl ester | 42.69 $^{**}$ | 10.27 $^{ns}$ | 9.52 $^{ns}$ |
| 31 | 9,12,15-octadecatrienoic acid methyl ester | 17.56 $^{**}$ | 0.004 $^{ns}$ | 0.14 $^{ns}$ |
| | **Acids** | | | |
| 32 | Acetic acid | 8.45 $^{ns}$ | 8.23 $^{ns}$ | 2.83 $^{ns}$ |
| 33 | Propanoic acid | 24.16 $^{*}$ | 0.68 $^{ns}$ | 0.26 $^{ns}$ |
| 34 | Dimethyl propanedioic acid | <0.01 $^{ns}$ | 199.83 $^{**}$ | 7.47 $^{ns}$ |
| 35 | Butanoic acid | 807.58 $^{**}$ | 1613.57 $^{**}$ | 921.46 $^{**}$ |
| 36 | 3-Methyl butanoic acid | | | 28.04 $^{ns}$ |
| 37 | Pentanoic acid | 0.40 $^{ns}$ | 0.17 $^{ns}$ | 0.03 $^{ns}$ |
| 38 | 4-Methyl-pentanoic acid | 1.48 $^{**}$ | 0.60 $^{*}$ | 0.007 $^{ns}$ |
| 39 | Hexanoic acid | 10.11 $^{*}$ | 0.28 $^{ns}$ | 5.64 $^{ns}$ |
| 40 | Heptanoic acid | <0.01 $^{ns}$ | 0.25 $^{ns}$ | 0.04 $^{ns}$ |
| 41 | Octanoic acid | 2.18 $^{*}$ | 5.98 $^{**}$ | 0.13 $^{ns}$ |
| 42 | Nonanoic acid | 12.29 $^{ns}$ | | 0.70 $^{ns}$ |
| 43 | Decanoic acid | 17.56 $^{ns}$ | 636.48 $^{**}$ | 16.91 $^{ns}$ |
| 44 | Benzoic acid | 0.57 $^{**}$ | 0.02 $^{ns}$ | 0.02 $^{ns}$ |
| 45 | Dodecanoic acid | 60.50 $^{**}$ | 30.60 $^{**}$ | 8.80 $^{*}$ |
| 46 | Tetradecanoic acid | 0.85 $^{ns}$ | 0.36 $^{ns}$ | 1.58 $^{ns}$ |
| 47 | Pentadecanoic acid | 0.10 $^{ns}$ | 0.02 $^{ns}$ | 0.08 $^{ns}$ |
| 48 | Hexadecanoic acid | 275.10 $^{**}$ | 5.10 $^{ns}$ | 112.94 $^{*}$ |
| | **Furans** | | | |
| 49 | Dihydro-2-methyl-3(2H)-furanone | 0.48 $^{*}$ | 0.22 $^{ns}$ | 0.14 $^{ns}$ |
| 50 | 5-ethenyltetrahydro-α,α-5-trimethyl-2-furanmethanol | 26.55 $^{**}$ | 2.49 $^{ns}$ | 1.43 $^{ns}$ |
| 51 | Furfural | 4.21 $^{**}$ | <0.01 $^{ns}$ | 0.55 $^{ns}$ |
| 52 | 1-(2-Furanyl) ethanone | 62.08 $^{**}$ | 0.11 $^{ns}$ | 1.14 $^{ns}$ |
| 53 | 5-Methyl-2-Furancarboxaldehyde | 135.60 $^{*}$ | 106.53 $^{**}$ | 120.63 $^{**}$ |
| 54 | Dihidro-5-methyl-2(3H)-furanone | 0.07 $^{ns}$ | 0.04 $^{ns}$ | 0.09 $^{ns}$ |
| 55 | 2-Furanmethanol | 0.09 $^{ns}$ | | 0.38 $^{ns}$ |
| 56 | 4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid | 43.51 $^{**}$ | 17.01 $^{**}$ | 77.69 $^{**}$ |
| | **Ketones** | | | |
| 57 | Cyclopentanone | 0.06 $^{ns}$ | | 0.07 $^{ns}$ |
| 58 | 3-Hidroxy-2-butanone | 0.26 $^{ns}$ | 0.45 $^{ns}$ | 0.18 $^{ns}$ |
| 59 | 3-Ethyl-cyclopentanone | <0.01 $^{ns}$ | | 0.01 $^{ns}$ |

**Table 1.** *Cont.*

| Compound Number | Compound Name | Agave Species | Origins | Seasons |
|---|---|---|---|---|
| 60 | 3,4-Dimethyl-2-cyclopenten-1-one | 0.23 * | 0.18 $^{ns}$ | 0.01 $^{ns}$ |
| 61 | 3-Methyl-2-cyclopenten-1-one | 0.04 $^{ns}$ | 0.88 ** | 0.17 * |
| 62 | 2,3-dimethyl-2-cyclopenten-1-one | 0.54 $^{ns}$ | 4.60 * | 0.60 $^{ns}$ |
| 63 | 1-(1 H-pyrrol-2-yl) ethanone | 0.15 $^{ns}$ | <0.01 $^{ns}$ | 0.05 $^{ns}$ |
| | **Phenols** | | | |
| 64 | 2-Methoxy phenol | 15.52 ** | 27.70 ** | 6.74 ** |
| 65 | Phenol | 217.95 * | 48.61 $^{ns}$ | 37.60 $^{ns}$ |
| 66 | 2 Ethyl-phenol | 3.05 * | 0.42 $^{ns}$ | 0.25 $^{ns}$ |
| 67 | 4-Methyl-phenol, *o p*-cresol | 12.01 $^{ns}$ | 56.72 * | 13.33 $^{ns}$ |
| 68 | 3-Methyl-phenol, *o m*-cresol | 24.65 ** | 3.61 $^{ns}$ | 2.83 $^{ns}$ |
| 69 | 2-Ethyl-6-methyl-phenol | 0.99 ** | 0.07 $^{ns}$ | 0.17 $^{ns}$ |
| 70 | 2-Methoxy-3-(2-propenyl)-phenol | 9.63 * | 37.81 ** | 1.87 $^{ns}$ |
| 71 | 4-Ethyl-phenol | 3.64 ** | 0.45 $^{ns}$ | 0.14 $^{ns}$ |
| 72 | 3-Ethyl-phenol | | 1.61 ** | 0.49 * |
| | **Terpens** | | | |
| 73 | Linalool | 58.16 ** | 2.46 $^{ns}$ | 2.93 $^{ns}$ |
| 74 | α-Terpineol | 584.34 ** | 8.54 $^{ns}$ | 7.76 $^{ns}$ |
| 75 | Farnesol | 0.03 $^{ns}$ | <0.01 $^{ns}$ | 0.35 $^{ns}$ |
| | **Others** | | | |
| 76 | Pyridine | 0.46 $^{ns}$ | | 0.01 $^{ns}$ |
| 77 | 1,1,3-Triethoxy propane | 1.61 ** | 2.13 ** | 1.23 ** |
| 78 | Benzaldehyde | 0.12 $^{ns}$ | 0.04 $^{ns}$ | 0.01 $^{ns}$ |
| 79 | Naphthalene | 1.35 ** | 0.45 $^{ns}$ | 0.06 $^{ns}$ |
| 80 | 1,8-Dimethyl naphthalene | 10.56 ** | 9.42 ** | 12.87 ** |
| 81 | 2,6-Dimethyl naphthalene | 1.17 ** | 0.01 $^{ns}$ | 0.02 $^{ns}$ |
| 82 | Acenaphthylene | 0.21 $^{ns}$ | 1.21 * | 0.95 * |
| 83 | Fluorene | 0.49 $^{ns}$ | 0.02 $^{ns}$ | 0.40 $^{ns}$ |
| 84 | Phenantrene | 0.02 $^{ns}$ | 11.32 ** | 0.59 * |

$^{ns}$ Not significant at $p > 0.05$; * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$.

**Table 2.** Mean concentration values for volatile compounds in mezcal by *Agave*, origin, and season [a].

| No. | Compound Name | KI [b] ($t_R$) | *A. angustifolia* | | | | | | | | *A. potatorum* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tlacolula | | | | Matatlán | | | | Sola de Vega |
| | | | S | F | W | TM | S | F | MM | T | |
| | **Alcohols** | | | | | | | | | | |
| 1 | 2-Methyl-1-propanol | (5.8) | 6.1 | 6.3 | 0.2 | 4.2 | 1.4 | 1.2 | 1.3 | 2.7 | 8.8 |
| 2 | 1-Butanol | (7.6) | 1.1 | 0.9 | - | 0.9 | 0.2 | 0.4 | 0.3 | 0.6 | 0.7 |
| 3 | 3-Methyl-1-butanol | (10.4) | 413.9 | 481.1 | 232.0 | 375.7 | 332.0 | 338.7 | 335.4 | 355.5 | 504.5 |
| 4 | 3-Methyl-3-buten-1-ol | (11.8) | 1.4 | 1.1 | 0.3 | 0.9 | 0.4 | 0.6 | 0.5 | 0.7 | 1.3 |
| 5 | Cyclopentanol | (13.9) | 2.9 | 2.8 | 0.5 | 2.1 | 0.4 | 0.5 | 0.45 | 1.26 | 1.5 |
| 6 | 4-Methyl-1-pentanol | (14.5) | 0.3 | 0.3 | 0.2 | 0.3 | 0.1 | 0.2 | 0.2 | 0.2 | 0.5 |
| 7 | 3-Methyl-1-pentanol | (15) | 0.4 | 0.6 | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.7 |
| 8 | 3-Methyl-cyclopentanol | (15.5) | 4.5 | 4.1 | 1.4 | 3.3 | 0.6 | 0.4 | 0.5 | 1.9 | 1.4 |
| 9 | 1-Hexanol | (16.2) | 3.2 | 3.5 | 1.4 | 2.7 | 1.7 | 1.3 | 1.5 | 2.1 | 2.5 |
| 10 | 3-Ethoxy-1-propanol | (17.1) | 2.4 | 2.3 | 2.7 | 2.5 | 1.1 | 1.1 | 1.1 | 1.8 | 6.0 |
| 11 | 2,3-Butanediol | 307 | 1.5 | 1.8 | 10.4 | 4.6 | 4.9 | 15.6 | 10.2 | 7.4 | 3.4 |
| 12 | 1-Octanol | 326 | 0.5 | 0.9 | 0.7 | 0.7 | 0.8 | 1 | 0.9 | 0.8 | 0.7 |
| 13 | Decanol | 526 | 0.3 | 0.4 | 0.3 | 0.3 | 1.0 | 1.7 | 1.4 | 0.8 | 0.8 |
| 14 | Benzyl alcohol | 635 | 4.5 | 2.5 | 1.5 | 2.8 | 1.7 | 1.1 | 1.4 | 2.1 | 3.2 |
| 15 | Phenylethyl alcohol | 668 | 104.9 | 81.7 | 109.5 | 98.7 | 117.8 | 84.8 | 101.3 | 100.0 | 79.9 |
| 16 | Benzene propanol | 792 | 0.9 | 0.8 | 0.6 | 0.8 | - | - | - | 0.8 | |
| 17 | 1-Hexadecanol | 1044 | 0.9 | 2.2 | 2.3 | 1.8 | 3.9 | 4.4 | 4.2 | 2.9 | 2.2 |
| | **Esters** | | | | | | | | | | |
| 18 | 3-Methyl-1-butanol acetate | (7) | 0.6 | 0.8 | 0.5 | 0.6 | 0.4 | 1.5 | 0.9 | 0.8 | 1.8 |
| 19 | Hexanoic acid ethyl ester | (11.1) | 0.3 | 0.5 | 0.5 | 0.4 | 1.2 | 2.6 | 1.9 | 1.2 | 0.6 |
| 20 | 2-Hidroxy propanoic acid ethyl ester | (15.9) | 95.5 | 92.3 | 61.4 | 83.1 | 29.7 | 8.5 | 19.1 | 51.1 | 32.4 |
| 21 | Octanoic acid ethyl ester | (19.6) | 2.5 | 3.4 | 5.2 | 3.7 | 12.9 | 21.3 | 17.1 | 10.4 | 4.7 |
| 22 | Decanoic acid ethyl ester | 408 | 2.6 | 3.7 | 6.6 | 4.3 | 24.5 | 33.2 | 28.8 | 16.6 | 8.4 |
| 23 | Butanedioic acid diethyl ester | 450 | 1.1 | 2.1 | 2.3 | 1.8 | 3.1 | 0.9 | 2.0 | 1.9 | 2.3 |
| 24 | Acetic acid 2 phenylethyl ester | 576 | 7.5 | 5.6 | 8.4 | 7.2 | 4.9 | 17.7 | 11.3 | 9.2 | 14.8 |
| 25 | Dodecanoic acid ethyl ester | 596 | 2.0 | 2.2 | 3.7 | 2.6 | 24.1 | 9.6 | 16.8 | 9.7 | 5.2 |
| 26 | 2-Cyclohexene-1-acetic acid, 1-hydroxy ethyl ester | 606 | 2.3 | 3.2 | 2.4 | 2.6 | 4.2 | 1.2 | 2.7 | 2.7 | 5.8 |

**Table 2.** *Cont.*

| No. | Compound Name | KI [b] ($t_R$) | *A. angustifolia* | | | | | | | | *A. potatorum* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tlacolula | | | | Matatlán | | | | Sola de Vega |
| | | | S | F | W | TM | S | F | MM | T | |
| 27 | Tetradecanoic acid ethyl ester | 787 | 1.0 | - | 1.1 | 1.1 | 9.1 | 4.1 | 6.6 | 3.8 | 5.6 |
| 28 | Hexadecanoic acid ethyl ester | 979 | 5.2 | 2.0 | 2.7 | 3.3 | 31.5 | 2.7 | 17.1 | 10.2 | 11.8 |
| 29 | (R) 2-Butenedioic acid diethyl ester | 1068 | 2.3 | 1.0 | 0.8 | 1.4 | 2.2 | 1.0 | 1.6 | 1.5 | 2.3 |
| 30 | 9,12-octadecadienoic acid ethyl ester | 1216 | 3.1 | 1.4 | 3.2 | 2.6 | 7.1 | 2.2 | 4.6 | 3.6 | 6.7 |
| 31 | 9,12,15-octadecatrienoic acid methyl ester | 1245 | 0.5 | 0.3 | 0.7 | 0.5 | 0.8 | 0.3 | 0.5 | 0.5 | 2.6 |
| | **Acids** | | | | | | | | | | |
| 32 | Acetic acid | 200 | 55.6 | 50.6 | 24.1 | 43.4 | 29.9 | 13.3 | 21.6 | 32.5 | 52.7 |
| 33 | Propanoic acid | 300 | 3.2 | 3.3 | 2.5 | 3.0 | 2.4 | 2.6 | 2.5 | 2.7 | 5.4 |
| 34 | Dimethyl propanedioic acid | 332 | 14.6 | 14.5 | 10.7 | 13.3 | 4.9 | 3.4 | 4.2 | 8.7 | 9.6 |
| 35 | Butanoic acid | 400 | 59.0 | 24.9 | 7.3 | 30.4 | 4.2 | 4.7 | 4.4 | 17.4 | 5.3 |
| 36 | 3-Methyl butanoic acid | 437 | 28.8 | 34.0 | 36.0 | 32.9 | - | - | - | 32.9 | |
| 37 | Pentanoic acid | 500 | 1.4 | 0.9 | 1.0 | 1.1 | 2.0 | 1.6 | 1.8 | 1.4 | 2.3 |
| 38 | 4-Methyl-pentanoic acid | 556 | 1.0 | 0.9 | 0.8 | 0. 9 | 0.4 | - | 0.4 | 0.6 | 2.2 |
| 39 | Hexanoic acid | 600 | 2.5 | 2.9 | 3.3 | 2.9 | - | 4.9 | 4.9 | 3.9 | 4.7 |
| 40 | Heptanoic acid | 700 | 1.9 | 1.4 | 2.0 | 1.7 | 3.2 | 2.4 | 2.8 | 2.3 | 2.2 |
| 41 | Octanoic acid | 800 | 9.9 | 12.4 | 9.2 | 10.5 | 26.2 | 21.4 | 23.8 | 17.2 | 9.9 |
| 42 | Nonanoic acid | 900 | 2.7 | 2.4 | 3.5 | 2.8 | - | - | - | 2.8 | 4.4 |
| 43 | Decanoic acid | 1000 | 9.5 | 15.8 | 12.8 | 12.7 | 27.3 | 30.7 | 29.0 | 20.8 | 21.4 |
| 44 | Benzoic acid | 1075 | 0.9 | 0.5 | 0.6 | 0.7 | 0.6 | 0.4 | 0.5 | 0.6 | 1.4 |
| 45 | Dodecanoic acid | 1200 | 6.7 | 11 | 11 | 9.6 | 12.7 | 13.6 | 13.2 | 11.4 | 7.0 |
| 46 | Tetradecanoic acid | 1400 | 2.7 | 3.6 | 4.7 | 3. 7 | 3.6 | 2.9 | 3.2 | 3.5 | 3.0 |
| 47 | Pentadecanoic acid | 1500 | 0.2 | 0.3 | 0.7 | 0.4 | 0.4 | 0.3 | 0.3 | 0.4 | 0.5 |
| 48 | Hexadecanoic acid | 1600 | 1.8 | 2.5 | 17.6 | 7.3 | 8.0 | 3.7 | 5.8 | 6.6 | 15.3 |
| | **Furans** | | | | | | | | | | |
| 49 | Dihydro-2-methyl-3(2H)-furanone | (12.4) | 1.1 | 0.5 | 0.2 | 0.6 | 0.2 | 0.1 | 0.1 | 0.4 | 1.0 |
| 50 | 5-ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol | 227 | 1.5 | 2.6 | 0.5 | 1.5 | 0.6 | 0.4 | 0.5 | 1.0 | 3.8 |
| 51 | Furfural | 232 | 2.9 | 1.8 | 0.4 | 1.7 | 2.5 | 0.7 | 1.6 | 1.6 | 5.4 |
| 52 | 1-(2-Furanyl) ethanone | 271 | 3.5 | 2.4 | 2.8 | 2.9 | 3.9 | 2.4 | 3.1 | 3.0 | 7.0 |
| 53 | 5-Methyl-2-Furancarboxaldehyde | 347 | 9.0 | 5.2 | 4.8 | 6.3 | 22.2 | 3.8 | 13.0 | 9.7 | 15.0 |
| 54 | Dihidro-5-methyl-2(3H)-furanone | 380 | 1.2 | 0.8 | 1.1 | 1.0 | 1.0 | 0.7 | 0.8 | 0.9 | 1.1 |
| 55 | 2-Furanmethanol | 439 | - | - | - | - | 18.3 | 13.5 | 15.9 | 15.9 | 18.1 |
| 56 | 4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid | 849 | 4.4 | 14.4 | 2.9 | 7.2 | 2.8 | - | 2.8 | 5.0 | 1.3 |
| | **Ketones** | | | | | | | | | | |
| 57 | Cyclopentanone | (9) | 0.6 | 0.3 | | 0.4 | - | - | - | 0.4 | 0.3 |
| 58 | 3-Hidroxy-2-butanone | (13.1) | 3.0 | 2.0 | 0.9 | 1.9 | 0.7 | 0.9 | 0.8 | 1.4 | 2.4 |
| 59 | 3-Ethyl-cyclopentanone | (14.8) | 1.1 | 1.0 | 0.9 | 1.0 | - | - | - | 1.0 | 1.0 |
| 60 | 3,4-Dimethyl-2-cyclopenten-1-one | 238 | 0.8 | 0.6 | 0.6 | 0.7 | 0.4 | 0.4 | 0.4 | 0.5 | 0.8 |
| 61 | 3-Methyl-2-cyclopenten-1-one | 276 | 1.3 | 0.6 | 0.7 | 0.8 | 0.3 | 0.2 | 0.2 | 0.6 | 0.7 |
| 62 | 2,3-dimethyl-2-cyclopenten-1-one | 295 | 2.9 | 1.7 | 2.1 | 2.2 | 1.1 | 0.6 | 0.8 | 1.5 | 2.1 |
| 63 | 1-(1 H-pyrrol-2-yl) ethanone | 756 | 0.7 | 0.3 | 0.8 | 0.6 | 0.7 | 0.5 | 0.6 | 0.6 | 1.0 |
| | **Phenols** | | | | | | | | | | |
| 64 | 2-Methoxy phenol | 618 | 7.4 | 4.5 | 3.0 | 4.9 | 1.5 | 1.7 | 1.6 | 3.3 | 1.6 |
| 65 | Phenol | 756 | 19.9 | 10.1 | 12.9 | 14.3 | 11.5 | 8.1 | 9.8 | 12.0 | 20.1 |
| 66 | 2 Ethyl-phenol | 820 | 1.5 | 0.9 | 0.9 | 1.1 | 0.4 | - | 0.4 | 0.7 | 2.0 |
| 67 | 4-Methyl-phenol, *o p*-cresol | 830 | 12.7 | 7.0 | 8.3 | 9.3 | 5.6 | 3.3 | 4.4 | 6.9 | 9.2 |
| 68 | 3-Methyl-phenol, *o m*-cresol | 837 | 5.3 | 2.7 | 3.3 | 3.7 | 3.1 | 2.0 | 2.5 | 3.2 | 5.8 |
| 69 | 2-Ethyl-6-methyl-phenol | 882 | 1.9 | 1.1 | 1.1 | 1.4 | 1.8 | 0.5 | 1.1 | 1.3 | 2.7 |
| 70 | 2-Methoxy-3-(2-propenyl)-phenol | 908 | 4.2 | 3.9 | 2.4 | 3.5 | - | 10.1 | 10.1 | 6.8 | 2.5 |
| 71 | 4-Ethyl-phenol | 917 | 4.6 | 2.8 | 3.8 | 3.7 | 3.0 | 1.6 | 2.3 | 3.0 | 7.7 |
| 72 | 3-Ethyl-phenol | 922 | 2.2 | 1.2 | 0.9 | 1.4 | 0.5 | 0.4 | 0.4 | 0.9 | - |
| | **Terpens** | | | | | | | | | | |
| 73 | Linalool | 315 | 1.1 | 4.0 | 2.9 | 2.7 | 1.5 | 1.9 | 1.7 | 2.2 | 6.2 |
| 74 | α-Terpineol | 462 | 4.6 | 8.6 | 4.5 | 5.9 | 4.6 | 3.4 | 4.0 | 4.9 | 17.6 |
| 75 | Farnesol | 1035 | 4.3 | 1.0 | 1.4 | 2.2 | 1.2 | 2.4 | 1.8 | 2.0 | 2.1 |
| | **Others** | | | | | | | | | | |
| 76 | Pyridine | (9.3) | 0.2 | 0.1 | 0.1 | 0.1 | - | - | - | 0.1 | 0.6 |
| 77 | 1,1,3-Triethoxy propane, | (14.1) | 4.0 | 9.1 | 1.2 | 4.7 | 1.0 | 1.4 | 1.2 | 2.9 | 0.9 |
| 78 | Benzaldehyde | 284 | 0.3 | 0.3 | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 | 0.5 |
| 79 | Naphthalene | 493 | 0.5 | 0.4 | 1.0 | 0.6 | 1.8 | 1.4 | 1.6 | 1.1 | 2.5 |
| 80 | 1,8-Dimethyl naphthalene | 1972 | 6.3 | 3.8 | 1.3 | 3.8 | - | - | - | 3.8 | 1.1 |
| 81 | 2,6-Dimethyl naphthalene | 2007 | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 | 0.2 | 0.35 | 0.4 | 1.5 |
| 82 | Acenaphthylene | 937 | 1.2 | 1.3 | 1.9 | 1.5 | 3.0 | 1.4 | 2.2 | 1.8 | 2.1 |
| 83 | Fluorene | 1032 | 3.8 | 0.9 | 0.8 | 1.8 | 2.2 | 1.3 | 1.75 | 1.8 | 2.7 |
| 84 | Phenantrene | 1422 | 0.7 | 0.3 | 0.7 | 0.6 | 13.0 | 5.2 | 9.1 | 4.8 | 2.5 |

[a]: Concentration of compounds in mg/L; [b]: Kovats index based on short and long fatty acids; $t_R$: retention time; -: not detected; S: spring; F: fall; W: winter; TM: mean value of Tlacolula mezcal; MM: mean value of Matatlán mezcal; T: mean value of *A. angustifolia* mezcal; SV: mean value of *A. potatorum* mezcal from Sola de Vega.

According to the variance analysis, twenty-six volatile compounds were significantly different ($p \leq 0.01$) based on the *Agave* species (Table 1). For example, 5-ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol, furfural, 1-(2-furanyl) ethanone, and 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid could be produced by a Maillard reaction during the cooking of the agave. Mancilla-Margalli and López [16] quantified these compounds at different cooking times of *A. tequilana* Weber and attributed their generation to Maillard reactions. Terpenes, such as linalool and α-terpineol, as well as fatty acids, including dodecanoic acid and hexadecanoic acid, likely come from the *Agave* plant per se, and the quantity of these compounds could be different in each *Agave* species [13,14]. Fatty acids form a minor yet important group in alcoholic beverages, giving specific odor and taste profiles to each drink [14,18]. These compounds could be used to understand the effect of agave species on different flavors of mezcal. In this study, it was possible to find variations between the amounts and numbers of compounds identified in each mezcal type (Table 2). Nonanoic acid was detected in mezcal made from *A. angustifolia* in Tlacolula and mezcal made from *A. potatorum* in Sola de Vega. Furthermore, 2-furanmethanol was found in mezcal made from *A. angustifolia* in Matatlan and *A. potatorum* in Sola de Vega. Concentration variation of these compounds for each type of mezcal varies depending on the cooking conditions of the agave. In *A. tequilana* Weber, it was reported that nonanoic acid levels decreased while 2-furanmethanol levels increased with cooking time [16]. However, benzenepropanol and 3-methyl butanoic acid were found only in mezcal made from *A. angustifolia* in Tlacolula, although these compounds could be related to the fermentation and distillation processes, respectively, as occurs in tequila production [12]. On the other hand, 3-ethyl-phenol was identified only in samples of mezcal made from *A. angustifolia*, and this volatile compound could be used as an authentic marker of mezcal made from *A. angustifolia*, which has not been reported in previous studies into mezcal from other agave species [2,18–21].

### *3.2. Volatile Compounds in Mezcal from A. angustifolia by Place of Origin and Season*

Volatile compounds identified in mezcal made from *A. angustifolia* were analyzed based on their community of origin and the production season. Using ANOVA, significant differences were determined ($p \leq 0.01$) in the concentrations of twenty-four volatile compounds between mezcal made in Tlacolula and Matatlan (Table 1). Most compounds were alcohols, esters and acids. The differences in the volatile compounds, such as benzyl alcohol, hexanoic acid ethyl ester, and dodecanoic acid ethyl ester concentrations, could be attributable to the microorganisms and conditions found during the fermentation process [8,9,22]. In previous studies, Kirchmayr et al. [10] identified 21 different yeast species, such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Zygosaccharomyces rouxii*, *Z. bisporus*, *Torulaspora delbrueckii*, and *Pichia membranifaciens*, and 27 different bacterial species during fermentation in artisan mezcal production. These were associated with volatile compound generation. In yeast strains isolated from *Agave tequilana Weber* juice, *S. cerevisiae* strains produced predominantly amyl and isoamyl alcohols, n-propanol, 2-phenyl ethanol, methanol, isoamyl acetate, ethyl hexanoate [22]. Changes in octanoic acid ethyl ester, decanoic acid ethyl ester, decanoic acid, and butanoic acid concentrations have also been reported in beer fermentation [23] and in wine fermentation, as a result of the yeast's response to nitrogen availability [24]. Furthermore, ten volatile compounds were significantly different ($p \leq 0.01$) between the spring, fall, and winter seasons. A similar pattern was observed in alcohols, esters, and acid compounds. Variations in these volatile groups among the three production seasons show an impact of microbial diversity, in addition to environmental factors, such as origin, ambient temperature and the traditional practices employed at each artisan distillery. Moreover, butanedioic acid diethyl ester and hexadecanoic acid were unique compounds that marked the differences between the production seasons of mezcal from *A. angustifolia* (Table 1).

The modification of the volatile compounds profile showed the effect of *Agave* species, place of origin, and season on the chemical composition of mezcal, which is why it could explain the diversity in the taste of artisanal mezcal made from *A. angustifolia* and *A. potatorum.* The presence of minor volatile compounds is relevant because they can harmonically synergize to produce the characteristic flavor and aroma of mezcal.

### *3.3. Aromatic Compounds of Mezcal Made from A. angustifolia and A. potatorum*

The aromatic alcohol fraction was formed by hexanol, octanol, decanol, phenylethyl alcohol, and hexadecanol compounds, which were detected in all products (Table 2). It has been reported that phenylethyl alcohol imparts a desirable aroma to alcoholic beverages, such as tequila [18]. Among the minor volatile compounds detected in mezcals, the most abundant compound was 3-methyl-1-butanol, and its excessive concentration could be responsible for the characteristic malty, alcohol, and vinous aromas in mezcals [18,25].

Regarding the ester family, ethyl esters from C6 to C16 were detected in all mezcal samples. Vertrespen et al. [26] mention that volatile esters are the product of an enzyme-catalyzed condensation reaction between acyl-CoA and a higher alcohol. It has been reported that esters are responsible for the presence of fruity-like aromas in alcoholic beverages [25]. These same esters may also contribute to the pleasant aroma of mezcals from each place of origin, which are preferred by the consumers given their sensory characteristics.

An abundant group of fatty acids, which varied from C2 (acetic acid) to C16 (hexadecanoic acid), were found in all evaluated samples with the exception of nonanoic acid, which was not present in any mezcal sample from Matatlán. Some fatty acids could be produced during alcoholic fermentations and are synthesized in the membrane structures during cell growth. They can also appear at the end of the fermentation process, when lysis occurs [27]. Other fatty acids, such as C10 (decanoic acid) to C16 (hexadecanoic acid), may stem from the *Agave* plant [14]. In this study, certain furans, such as furfural and 5-methyl furfural, were also found. These pleasantly aromatic compounds may also originate during the cooking of *Agave* [16]. In terms of furanoic compounds, only 2-furanmethanol was not detected in mezcals produced from *A. angustifolia* Haw. of Tlacolula.

Additionally, a significant presence of volatile phenols, such as *o*-cresol (*p*-cresol and *m*-cresol) and *p*-ethyl phenol, were detected in all samples. It is interesting to observe that these compounds have not been reported in mezcal [7,19,21]. Volatile phenolic derivatives have been associated with smoked foods and certain characteristics of wines [25]. However, phenol and phenol derivatives may be formed by the thermal degradation of lignin during the cooking process to produce mezcal [16].

Other volatile families, such as terpenoids, aldehydes, naphthalenes and polycyclic aromatic hydrocarbons, were also detected. Terpenoids, such as linalool and α-terpineol, were detected in all mezcal samples. These terpenoids have been reported previously in spirits made from different *Agave* species; however, a new compound in mezcals, farnesol, was detected. Terpenoids have particularly desirable floral notes [25] that could contribute to the overall flavor of mezcals.

## 4. Conclusions

According to these results, it is clear that the composition of the aroma of mezcal is extremely complex. Similarities and differences found between mezcal samples can be attributed to the conditions and the raw materials used, in addition to the origin and the production season. In this study, it was possible to find variations between the amounts and numbers of compounds identified in each mezcal type. The main volatile compounds were alcohols, esters and organic acids, as well as some phenols, ketones, furans, naphthalene, and others. 3-Ethyl-phenol was identified only in samples of mezcal made from *A. angustifolia*, and this volatile compound could be used as an authentic marker of mezcal made from *A. angustifolia*, which has not been reported in previous studies. For the first time, farnesol was identified in mezcals.

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