Behavior of the volatile compounds regulated by the Mexican Official Standard NOM-070-SCFI-2016 during the distillation of artisanal Mezcal

Comportamiento de los compuestos volátiles regulados por la Norma Oficial Mexicana NOM-070-SCFI-2016 durante la destilación de Mezcal artesanal

Nolasco-Cancino H1, Jarquín-Martínez D1, Ruiz-Terán F2, Santiago-Urbina JA3*
1 Facultad de Ciencias Químicas, Universidad Autónoma Benito Juárez de Oaxaca, Av. Universidad S/N, CP 68120, Ex-Hacienda S Señores, Oaxaca de Juárez, México.
2 Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Ciudad de México, México
3 Dirección de División de Carrera de Agricultura Sustentable y Protegida, Universidad Tecnológica de los Valles Centrales de Oaxaca, Villa de San Pablo Huixtepec, CP 71270, Zimatlán, Oaxaca, México.

RESUMEN
El mezcal se produce por fermentación del jugo de maguey y doble destilación en alambiques de cobre. Durante la segunda destilación, el destilado se separa en tres cortes o fracciones: cabeza, cuerpo y cola. El presente estudio tuvo como objetivo determinar el progreso de los compuestos volátiles regulados por la NOM-070-SCFI-2016 durante la primera y segunda destilación. Se recolectaron alícuotas directamente del flujo del destilado de un lote de mezcal artesanal y se analizaron por cromatografía de gases y densímetro digital. La fracción cabeza se colectó de 77.71 a 74.30 % (v/v) de etanol, la fracción cuerpo hasta 28.83 % (v/v), y la fracción cola hasta 14.44 % (v/v). El primer litro de cada fracción contenía 1731.86, 656.54 y 102.6 mg/100 mL a.a. de alcoholes superiores; 72.86, 35.37 y 1.77 mg/100 mL a.a. de aldehídos; 135.33, 142.95 y 247.6 mg/100 mL a.a. de metanol; 0.30, 0.45 y 3.04 mg/100 mL a.a. de furfural. Los alcoholes superiores, ésteres y aldehídos predominaron al inicio de la destilación, mientras que el furfural y el metanol prevalecen al final. Estos resultados serán de utilidad para los Maestros mezcaleros, contribuyendo a un mejor control de calidad del Mezcal.

Palabras clave: mezcal artesanal, destilación, compuestos volátiles, cortes de la destilación.

ABSTRACT
Mezcal is produced by the fermentation of maguey juice followed by a double distillation in copper stills. During the second distillation, three distillate cuts or fractions are separated: head, heart, and tail. The present study aimed to determine the progress of those volatile compounds regulated by the NOM-070-SCFI-2016 during the first and second distillation process. Aliquots were collected directly from the distillate flow of the artisanal Mezcal batch and analyzed by gas chromatography and digital densitometer. The head fraction was collected from 77.71 to 74.30 % (v/v) of ethanol, the heart fraction to 28.83 % (v/v), and the tail fraction collected until 14.44 % (v/v). The first liter of each fraction contained 1731.86, 656.54, and 102.6 mg/100 mL a.a. (anhydrous alcohol) of esters; 421.21, 452.28, and 40.26 mg/100 mL a.a. of higher alcohols; 72.86, 35.37, and 1.77 mg/100 mL a.a. of aldehydes; 135.33, 142.95, and 247.6 mg/100 mL a.a. of methanol; 0.30, 0.45, and 3.04 mg/100 mL a.a. of furfural. Higher alcohols, esters, and aldehydes predominate at the beginning of the distillation, while furfural and methanol prevail at the end. These results will be useful for the Maestros mezcaleros, contributing to a better quality control of Mezcal.

Keywords: artisanal Mezcal, distillation, volatile compounds, distillation cuts.

INTRODUCTION
Mezcal is a traditional Mexican spirit, it is obtained by distillation of fermented maguey juice and produced in the territory protected by the Appellation of Origin Mezcal (NOM-070-SCFI-2016). Mezcal is classified into three categories: Ancestral Mezcal, Artisanal Mezcal, and Mezcal (NOM-070-SCFI-2016). From these, Artisanal Mezcal is the most produced, with 86 % (6,747,775 L) of the total production (2021 statistical report; Mezcal Regulatory Council, 2021).

All categories of Mezcal must meet specific chemical parameters for its commercialization. According to the Mexican Official Standards NOM-070-SCFI-2016, Mezcal should have an alcohol concentration of 35 to 55 % by volume at 20°C; the higher alcohols and methanol concentrations must be within the limits of 100-500 mg/100 mL of anhydrous alcohol (a.a.) and 30-300 mg/100 a.a., respectively. The maximum limits permitted for aldehydes and furfural are 40 and 5 mg/100 mL a.a., respectively.

In Artisanal Mezcal production, piñas of maguey are cooked in a pit oven for 3 to 5 days, where the maguey fructans are hydrolyzed into fermentable sugars. Then, cooked maguey is milled using a tahona. After that, bagasse (pulp and fiber) obtained by the milling of cooked maguey is placed in 1000 L wooden vats, followed by the addition of...
water (Nolasco-Cancino et al., 2018); sugars in maguey must undergo a spontaneous fermentation. Finally, the fermented maguey juice is double distilled in 300 L copper still. Firstly, the copper boiler is filled with fermented juice plus bagasse in an approximately relation of 1.5:1. Subsequently, it is heated with direct flame fueled by regional wood or butane to obtain the first distillate named “simple”, “shishe” or “ordinario” which is then brought back to the boiler for a second distillation to obtain three distillate fractions: head, heart, and tail. These fractions are performed based on the experiences of the Maestros mezcaleros. They measure the alcohol content in the distillate using a traditional method, which consists of inspecting the lifetime of bubbles (regionally known as pearls) that are formed by a stream of the distillate poured into a “jícara” (small wooden cup, made from the bark of the Crescentia alata fruit). This empirical method helps measure ethanol concentrations close to 50 % v/v (Rage et al., 2020).

Also, distillation cuts are determined by smelling and tasting the distillate. The producers are guided by the herbaceous, fruity, and alcoholic aromas and flavors. In many cases, when producers are in the certification process and have not received training on the artisanal distillation process, mezcal can contain high levels of furfural, higher alcohols, and methanol mainly.

Some studies have been done to understand the distribution of volatile compounds during the distillation process in alcoholic beverages, such as plum brandies, Maotai liquor, spirit from Spine grape (Spaho et al., 2013; Balcerek et al., 2017; Cai et al., 2019; Xiang et al., 2020). These authors have reported that higher alcohols, aldehydes, and most esters predominate in the head fraction. While, in tail fraction, furfural and methanol occur in significant quantities (Balcerek et al., 2017). Although the absolute concentration of these volatile compounds varies between distillates, their behavior during the distillation process is the same. In the distillation of tequila and cocoy de pecaya (agave spirits), these volatile compounds also have the same behavior as in the spirits mentioned above (Prado-Ramirez et al., 2005; Granadillo et al., 2007). In Mezcal, no studies have been carried out that allow us to know the chemical compounds’ behavior throughout the artisanal distillation process.

The objective of this work was to determine the behavior of the ethanol, methanol, higher alcohols, esters, furfural, and aldehydes compounds during the first and second distillation processes. This information helps to provide recommendations to artisanal producers to efficiently control the distillation cuts and certify and mark their Mezcal.

MATERIALS AND METHODS

Sampling site

Samples of distillate were collected during the mezcal production process in Danzantes factory, an artisanal distillery located in Santiago Matatlán, Oaxaca, Mexico. Samples were taken during the distillation progress of a batch of artisanal Mezcal.

Fermentation

Mezcal was made by a Maestro mezcalero of the Danzantes distillery, following their traditional process. The batch of mezcal was produced using maguey espadín (Agave angustifolia). Cooked and crushed maguey pineapples (pulp and fiber) plus water were poured into a 1000 L wooden vat in a 60:40 (maguey: water) ratio. Fermentation was carried out naturally (no yeast addition) for approximately eight days.

Distillation and sample collection

First distillation

Immediately upon completion of fermentation, distillation was carried out in a 300 L copper still. The copper boiler was filled with 150 L of fermented maguey juice plus 100 kg of bagasse (pulp and fiber of the fermented maguey). It was heated with direct flame fueled by butane to obtain the first distillation named “ordinario”. This process was monitored in two stills to understand the behavior of normative compounds in the first distillation. During distillation, the flow rate was maintained approximately at 206 and 217 mL/min in each still. The first distillation was stopped according to the recommendation and experience of the Maestro mezcalero. The distillate is stored in food-grade plastic containers to be subjected to a second distillation subsequently.

A total of 24 aliquots were directly collected from the distillate flow of each still (in this study 2 stills were sampled). An aliquot consisted of a 100 mL sample for each liter of distillate. Samples were collected in 100 mL plastic bottles and stored at 4°C until analysis.

Second distillation

To know the behavior of the normative compounds in the second distillation and identify the distillate cuts, 250 L of ordinario were redistilled and fractionated in the head, heart, and tail. The ordinario was placed in the copper boiler and heated with direct flame fueled by butane. During distillation, the flow rate was maintained at approximately 170 mL/min. In this process, the Maestro mezcalero, according to his experience, made the distillate cuts (head, heart, and tail fractions). The head fraction consisted of the first 9 L distillate. Then, 86 L distillate were collected as the heart fraction. After that, the distillate was collected as the tail fraction comprised 12 L. Each fraction was stored in food-grade plastic containers until adjusting the alcoholic graduation and bottling.

In this stage, 109 aliquots were directly collected from the distillate flow of a single still. Samples 1 to 9 corresponded to the head fraction; samples 10 to 96 were collected from the heart fraction, and samples 97 to 109 were collected from the tail fraction. Samples were collected in 100 mL plastic bottles and stored at 4°C until analysis.

ANALYTICAL METHODS

Ethanol determination

Ethanol concentration was determined as indicated by the Mexican norm (NMX-V-013-NORMEX-2013) at 20°C, using an Anton Paar digital densitometer (Anton Paar, DMA...
RESULTS AND DISCUSSION

Mezcal has a complex volatile compounds composition which contributes to its organoleptic profile (Vera-Guzmán et al., 2018). During the distillation process, these compounds are separated from the must according to their boiling point, solubility in alcohol or water, variation of alcohol content in the vapor and liquid phases (Xiang et al., 2020), and by the distillation equipment employed (Balcerkek et al., 2017). Distillation is a critical stage where normative compounds can be controlled through appropriate distillate cuts.

Ethanol behavior

During the first distillation, ethanol and volatile compounds are separated from the fermented maguey must. Therefore, two products are obtained at this stage: a) the distillate named ordinario and b) vinasses or waste.

The behavior of the ethanol concentration and volatile compounds was similar between stills (no statistical differences were found with t-test, p≤0.05). Figure 1a shows the behavior of ethanol during the first distillation process. The ethanol concentration was 45.34±5.07 % (v/v) in the first liter of distillate. This alcoholic strength decreased during the distillation progress. Thus, the last liter of the ordinario sample collected (sample 24) had an ethanol concentration of 12.61±1.71 % (v/v). In artisanal distilleries, the producer defines the volume of ordinario collected based on their

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ethanol (g L⁻¹)</th>
<th>Acetaldehyde (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.23</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>1.18</td>
<td>0.52</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>24</td>
<td>0.56</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Figure 1. Behavior of ethanol and acetaldehyde during the first (a) and second (b) distillation of artisanal Mezcal. The vertical dotted lines on figure “b” indicate the distillate cuts. In figure “a”, concentration values are given as the mean of the samples from two copper stills ± standard deviation.

Figura 1. Comportamiento del etanol y acetaldéído durante la primera (a) y segunda (b) destilación del Mezcal artesanal. Las líneas punteadas verticales en la figura “b” indican los cortes del destilado. En la figura “a”, los valores de las concentraciones se dan como la media de las muestras de dos alambiques de cobre ± desviación estándar.
The ethanol content in the ordinario mainly depends on its concentration in the fermented must. It has been reported that ethanol concentration in maguey fermentation is approximately between 4 and 6 % v/v (Kirchmayr et al., 2017).

In the second distillation, the distillate was separated into three fractions: head, heart, and tails (Figure 1b). These fractions consisted of approximately 3.6 % (9 L), 34.4 % (86 L), and 4.8 % (12 L), respectively, of the base ordinario volume (250 L) placed in the boiler. The ethanol concentration in the head fraction ranged from 77.71 % (sample 1) v/v to 74.3 % v/v (sample 9; Figure 1b). Then, the heart fraction was collected from the samples 10 to 96, starting with 74.72 % (v/v) of ethanol, and its content was reduced as the distillation process progressed. The last liter of the heart fraction (sample 96) had an alcohol content of 30.39 % v/v (Figure 1b). After that, the distillate was collected as the tail fraction until sample 109, where the ethanol content was 14.44 % v/v (Figure 1b). The heart fraction was separated in other studies with spirits until alcohol content dropped under 55 % (v/v), and the tail fraction was collected until ethanol was lower than 30 % v/v (Xiang et al., 2020). In experiments with plum brandies, the heart’s fraction was cut until the alcohol reached a concentration of 40, 45, or 50 % v/v (Spaho et al., 2013), while in tequila it has been suggested to collect the heart fraction up to 35% (v/v) of ethanol (Prado-Ramírez et al., 2005). These distillate cuts will define the quality of the spirits because these may have more or less compounds regulated by the laws of each country.

The heart fraction is what becomes the finished product, and the producer adds drinking or demineralized water to adjust the alcohol content in the range established by the NOM-070-SCFI-2016 (35-55 % v/v at 20°C). According to the production practices in the distillery, some producers use a fair number of heads and tails to adjust the alcohol content and accentuate some aromas in the distillate. However, this practice frequently increases the concentration of some compounds regulated by Mexican standards, such as methanol and furfural (when tails are used), higher alcohols, and aldehydes (when heads are used). On the other hand, the most experienced Maestros mezcaleros redistilled the heads and tails fractions in another batch to collect more alcohol. Also, some producers use these distillation fractions to produce mixed beverages and distillates of lower quality.

**Aldehydes**

In spirit, some aldehydes such as isobutanal, 2-methylbutanal, and furfural are thermally formed during the first step of distillation (Awad et al., 2017). In contrast, others like acetaldehyde are formed during the lag and the onset of yeast growth phases and ethanol oxidation (Jackowetz et al., 2011). This metabolite in low concentration can give a fruity character to alcoholic beverages; nevertheless, in higher concentration, it provides a negative effect on sensory characteristics causing a pungent smell (Balcerek et al., 2017).

In Mezcal, acetaldehyde should not exceed 40 mg/100 mL of ethanol 100 % (v/v). This metabolite is quantitated according to the standard Mexican norm NMX-V-005-NORMEX-2013, which establishes that this compound is the most abundant of the aldehydes in alcoholic beverages, which is why only this is considered when reporting aldehydes. Although acetaldehyde is water-soluble, it has a boiling point of 20.4°C; therefore, it was found mainly at the beginning of the distillation process. In the first liter of ordinario, this metabolite had a concentration of 35.11±5.03 mg/100 mL a.a. (Figure 1a). This value rapidly decreased during the first nine liters of distillate, reaching a concentration of 9.64 mg/100 mL a.a., which remained almost constant during distillation progress (Figure 1a). It is probably by the solubility both in ethanol and water.

During the second distillation, acetaldehyde showed similar behavior to that of the first distillation (Figure 1b). However, the acetaldehyde content was higher in the head fraction (from 72.82 to 36.77 mg/100 mL a.a.) than in the heart and tail (Figure 1b). This result is according to the reported by Balcerek et al. (2017). The first liter of heart fraction had an acetaldehyde concentration of 35.37 mg/100 mL a.a. The last liter collected from this fraction had 1.72 mg/100 mL a.a. (Figure 1b). The tail fraction was characterized by the low content of this metabolite (2.39 to 3.87 mg/100 mL a.a.). Similar acetaldehyde concentration in the tail fraction was reported by Alcarde et al. (2011).

The results indicate that Mezcal (heart fraction) will comply with the permissible limit of aldehydes as established by the NOM-070-SCFI-2016.

**Higher alcohols**

Figure 2a shows the higher alcohols and esters concentrations obtained from the samples collected during the first distillation process. NOM-070-SCFI-2016 through NMX-V-005-NORMEX-2013 establishes that the higher alcohol concentration corresponds to the sum of 2-butanol, n-propanol, isoamyl alcohol, n-butanol, isoamyl alcohol, and amyl alcohol. Among them, isoamyl and isobutyl alcohols were present with the highest concentration (Table 1). These alcohols also were predominant in plum brandies (Spaho et al., 2013). However, in this spirit, 1-propanol has been reported as the principal higher alcohol (Spaho et al., 2013; Balcerek et al., 2017). This difference is probably due to the raw material used to produce each spirit, which can contain different amino acids and therefore promote the synthesis of different higher alcohols.

In general, higher alcohols were abundant at the beginning of the distillation. The first liter of ordinario had a concentration of 575.80±58.85 mg of higher alcohols/100 mL a.a. (Figure 2a), 95 % of them consisted of isobutylic and isoamyl alcohol (Table 1). Despite the high boiling temperature of these alcohols (isobutyl alcohol, 108°C; and isoamyl alcohol, 131°C), they were predominant in the heart fraction (Fisher LSD test, p<0.05). This is probably due to their low solubility in water (Léauté, 1990) and high affinity to ethanol,
forming azeotropic mixtures so that they can distill together with ethanol (Xiang et al., 2020). Isoamyl alcohol had the highest concentration throughout the entire distillation. This higher alcohol is synthesized by leucine metabolism through the Ehrlich route performed by yeast cells (Loviso and Libkind, 2019). Thus, the higher alcohol concentration in the distillate could be influenced by raw material (maguey species), yeasts, fermentation conditions, distillation techniques, experiences of the mezcal producers, and others.

In the second distillation, the head fraction was characterized by a high concentration of higher alcohols (450 mg/100 mL a.a., approximately, Figure 2b), mainly isobutyl and isoamyl alcohols (Table 2). The behavior of these alcohols was like that observed in the first distillation. The heart fraction started with a concentration of higher alcohols of 450 mg/100 mL a.a. (Figure 2b) and finished with a value of 50 mg/100 mL a.a. This information indicates that the batch of artisanal Mezcal will be within limits allowed by the Official Mexican standard (100 and 500 mg/100 mL of a.a.). The tail fraction had a lower concentration of higher alcohols (around 50 mg/100 mL a.a.). In general, the behavior of higher alcohols in artisanal Mezcal was similar to those reported in sugarcane spirits (Alcarde et al., 2011).

Some authors have demonstrated that higher alcohols are the largest group of aroma compounds in spirits (Spaho et al., 2013; Anjos et al., 2020).

![Figure 2. Behavior of higher alcohols and esters during the first (a) and second (b) distillation of artisanal Mezcal. The vertical dotted lines on figure “b” indicate the distillate cuts. In figure “a”, concentration values are given as the mean of the samples from two copper stills ± standard deviation.](image)

**Esters**

Although NOM-070-SCFI-2016 does not regulate esters, they are required by the laws of other countries to which Mezcal is exported. In Mexico, Tequila must meet the permissible limits for esters (as ethyl acetate). The standard Mexican NMX-V-005-NORMEX-2013 establishes that esters should be considered as the sum of ethyl acetate and ethyl lactate. At the beginning of the distillation, they were present at high concentrations (Figure 2a). The first sample of ordinario had an esters content of 491.88±5.81 mg/100 mL a.a. From the third sample (third liter of distillate), this concentration was reduced by approximately 70 %, reaching a concentration of 151.91±15.83 mg/100 mL a.a. Esters’ content decreased rapidly at the beginning of the distillation, which is attributed to the decrease in the ethyl acetate concentration. This metabolite was predominant (470 mg/100 mL a.a.) at the

**Table 1. Concentration of volatile compounds during the first distillation of mezcal.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ethyl acetate</th>
<th>Ethyl lactate</th>
<th>2-Butanol</th>
<th>n-propanol</th>
<th>Isobutyl alcohol</th>
<th>n-Butanol</th>
<th>Isoamyl alcohol</th>
<th>Amyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>470±7.59a</td>
<td>21.88±1.71h</td>
<td>0.88±0.15c</td>
<td>25.21±0.58c</td>
<td>107.06±9.39a</td>
<td>0.60±0.04ab</td>
<td>440.80±49.56a</td>
<td>1.26±0.32d</td>
</tr>
<tr>
<td>3</td>
<td>124.09±16.10b</td>
<td>27.82±0.28gh</td>
<td>0.81±0.03c</td>
<td>23.10±2.74a</td>
<td>81.49±5.26b</td>
<td>0.54±0.04b</td>
<td>319.69±16.77b</td>
<td>1.24±0.06d</td>
</tr>
<tr>
<td>5</td>
<td>70.26±5.38c</td>
<td>31.26±0.10g</td>
<td>0.83±0.03c</td>
<td>21.88±0.96c</td>
<td>66.73±1.65c</td>
<td>0.54±0.01b</td>
<td>258.49±4.68c</td>
<td>1.28±0.03d</td>
</tr>
<tr>
<td>7</td>
<td>64.95±0.73c</td>
<td>33.03±3.88g</td>
<td>0.88±0.05c</td>
<td>22.12±2.11c</td>
<td>58.20±6.50c</td>
<td>0.55±0.03b</td>
<td>241.35±32.58c</td>
<td>1.24±0.23d</td>
</tr>
<tr>
<td>10</td>
<td>28.49±5.97d</td>
<td>41.42±1.80h</td>
<td>0.82±0.02c</td>
<td>21.15±0.59c</td>
<td>46.36±0.86d</td>
<td>0.52±0.04b</td>
<td>163.67±4.38c</td>
<td>1.43±0.11d</td>
</tr>
<tr>
<td>12</td>
<td>20.47±1.73d</td>
<td>48.50±1.20e</td>
<td>0.89±0.10c</td>
<td>20.72±0.63c</td>
<td>41.45±1.78d</td>
<td>0.49±0.03b</td>
<td>146.47±16.78c</td>
<td>1.56±0.17d</td>
</tr>
<tr>
<td>15</td>
<td>31.79±19.58c</td>
<td>54.93±4.47d</td>
<td>0.98±0.04c</td>
<td>20.51±1.13c</td>
<td>30.38±2.10c</td>
<td>0.48±0.09b</td>
<td>89.01±4.58c</td>
<td>1.56±0.26c</td>
</tr>
<tr>
<td>17</td>
<td>31.06±17.58c</td>
<td>63.36±0.35c</td>
<td>0.97±0.11c</td>
<td>22.43±1.98c</td>
<td>29.70±0.11c</td>
<td>0.54±0.05b</td>
<td>87.84±8.1c</td>
<td>1.81±0.20c</td>
</tr>
<tr>
<td>20</td>
<td>27.97±24.46c</td>
<td>77.91±0.85c</td>
<td>1.06±0.07c</td>
<td>21.79±1.22c</td>
<td>28.24±0.61c</td>
<td>0.57±0.04b</td>
<td>78.63±3.91c</td>
<td>2.41±0.01b</td>
</tr>
<tr>
<td>24</td>
<td>23.35±20.01d</td>
<td>91.73±5.66a</td>
<td>1.41±0.10c</td>
<td>21.95±2.81c</td>
<td>25.70±3.00c</td>
<td>0.77±0.25a</td>
<td>63.14±6.34c</td>
<td>3.28±0.43c</td>
</tr>
</tbody>
</table>

Values of concentrations are given as the mean of two samples from two copper stills ± standard deviation. Different superscript letters in the same column show significant differences according to the analysis of variance at p ≤ 0.05 (Fisher LSD test).
beginning of the distillation (Table 1). Similar behavior of this ester has been reported in plum brandies, spirit from Spine grape (Spaho et al., 2013; Balcerek et al., 2017; Xiang et al., 2020). Its low boiling point (77°C) explains this and high solubility in ethanol (Léauté, 1990). On the other hand, esters’ content decreased slowly in the middle and at the end of the first distillation (Figure 2a). Although the ethyl acetate concentration decreased (Fisher LSD test, p≤0.05), the ethyl lactate concentration increased (Table 1). The initial concentration of ethyl lactate was 21.88±1.78 mg/100 mL a.a., which gradually increased to 91.73±5.68 mg/100 mL a.a. The behavior of this metabolite is due to its high boiling point (154°C) and its solubility in water (Léauté, 1990).

In the second distillation, the head fraction was characterized by a high concentration of esters (1731.86 to 883.77 mg/100 mL a.a.), mainly ethyl acetate (Table 2). Esters were present in the heart fraction, ranging from 656.64 to 95.48 mg/100 mL a.a. The ethyl acetate concentration decreased while the ethyl lactate increased (Table 2). Thus, the tail fraction consisted mainly of ethyl lactate. This behavior is due to the high boiling point of ethyl lactate (154°C) and its solubility in water (Léauté, 1990).

Methanol

Methanol is generated during maguey cooking by the demethoxylation of the pectins present in the agave plants (Solís-García et al., 2017). Immature maguey has been reported to have a higher pectin content than mature maguey (Pinal et al., 2009). Therefore, the use of mature maguey is recommended to decrease methanol generation. Keep the methanol concentration within the permissible limits by Mexican standards is the main challenge for artisan producers. Many producers assume that the highest methanol concentrations are in the head fraction, only considering its low boiling point (64.7°C). In this study, methanol was present throughout the distillation process, and it shows an increase towards the end of the distillation process. Thus, its initial concentration was 138.22±10.10 mg/100 mL a.a., this value increased constantly to 297.87±32.65 mg/100 mL a.a. (Figure 3a). This behavior of methanol is due to solubility in water and its capacity to form hydrogen bonds with this molecule, which increases its molecular weight and decreases its volatility (Balcerek et al., 2017).

In the second distillation, the methanol concentrations in the head and heart fractions were lower than those in the tail fraction (Figure 3b). The main fraction, the heart, had an initial methanol concentration of 148.71 mg/100 mL a.a., increasing progressively in each sample collected, reaching a concentration of 238.08 mg/100 mL a.a. in the last sample (Figure 3b). This compound occurred in concentrations lower than the limits specified by Mexican regulations in this fraction of distillate. Tail fraction was characterized by high methanol concentrations (239.11 to 362.16 mg/100 mL a.a. Figure 3b). This behavior of methanol was associated with the phenomenon described above.

Table 2. Concentration of volatile compounds during the second distillation of artisanal mezcal.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Distillate fraction</th>
<th>Compound concentration (mg/100 mL a.a.)</th>
<th>Ethyl acetate</th>
<th>Ethyl lactate</th>
<th>2-Butanol</th>
<th>n-Propanol</th>
<th>Iso-butyl alcohol</th>
<th>n-Butanol</th>
<th>Iso-amyl alcohol</th>
<th>Amyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Head</td>
<td>1726.82</td>
<td>5.04</td>
<td>1.39</td>
<td>25.95</td>
<td>99.78</td>
<td>0.22</td>
<td>293.23</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Head</td>
<td>999.67</td>
<td>7.98</td>
<td>1.57</td>
<td>26.00</td>
<td>93.96</td>
<td>0.46</td>
<td>296.48</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Head</td>
<td>874.95</td>
<td>8.82</td>
<td>1.63</td>
<td>29.27</td>
<td>105.16</td>
<td>0.54</td>
<td>335.88</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Heart</td>
<td>471.92</td>
<td>9.61</td>
<td>0.97</td>
<td>27.89</td>
<td>103.9</td>
<td>0.53</td>
<td>338.79</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Heart</td>
<td>162.53</td>
<td>9.66</td>
<td>0.88</td>
<td>24.89</td>
<td>88.13</td>
<td>0.49</td>
<td>309.64</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Heart</td>
<td>105.65</td>
<td>10.48</td>
<td>0.98</td>
<td>25.29</td>
<td>82.66</td>
<td>0.51</td>
<td>308.20</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Heart</td>
<td>38.45</td>
<td>12.21</td>
<td>1.03</td>
<td>24.15</td>
<td>67.72</td>
<td>0.41</td>
<td>280.75</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Heart</td>
<td>19.18</td>
<td>15.26</td>
<td>0.92</td>
<td>23.13</td>
<td>54.40</td>
<td>0.43</td>
<td>234.30</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Heart</td>
<td>18.27</td>
<td>22.00</td>
<td>0.89</td>
<td>26.59</td>
<td>51.68</td>
<td>0.50</td>
<td>240.59</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Heart</td>
<td>13.45</td>
<td>17.79</td>
<td>0.56</td>
<td>22.60</td>
<td>42.92</td>
<td>0.35</td>
<td>187.71</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>Heart</td>
<td>16.30</td>
<td>28.94</td>
<td>0.51</td>
<td>21.94</td>
<td>32.64</td>
<td>0.37</td>
<td>144.63</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>Heart</td>
<td>19.56</td>
<td>35.79</td>
<td>0.54</td>
<td>19.74</td>
<td>33.96</td>
<td>0.35</td>
<td>123.97</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>Heart</td>
<td>26.38</td>
<td>47.79</td>
<td>0.58</td>
<td>17.54</td>
<td>13.48</td>
<td>0.25</td>
<td>48.21</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Tail</td>
<td>27.39</td>
<td>75.21</td>
<td>0.50</td>
<td>13.00</td>
<td>9.40</td>
<td>0.29</td>
<td>15.32</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Tail</td>
<td>33.94</td>
<td>101.28</td>
<td>0.52</td>
<td>16.90</td>
<td>9.79</td>
<td>0.65</td>
<td>20.48</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Tail</td>
<td>44.85</td>
<td>128.88</td>
<td>0.60</td>
<td>17.95</td>
<td>9.53</td>
<td>0.97</td>
<td>29.89</td>
<td>2.37</td>
<td></td>
</tr>
</tbody>
</table>

The concentration values correspond to a single measurement of the samples collected from the distillate of the second distillation.
The hydrolysis of fructans by heat treatments also leads to the generation of furans such as 5-(hydroxymethyl)furfural, furfural, and others (García-Soto et al., 2011). These compounds are formed by the degradation of reducing sugars (García-Soto et al., 2011) and the Maillard reaction (Mancilla-Margalli and López, 2002). Also, furfural synthesis occurs in the heated pot (Balcerek et al., 2017), mainly during the first distillation (Awad et al., 2017).

The 5-HMF plays an essential role in the flavor of the Mezcal; this contributes to the characteristic aroma of cooked maguey. However, this compound and furfural hurt the yield of ethanol production; it affects yeast metabolism (consumption of sugars) (García-Soto et al., 2011; Mancilla-Margalli and López, 2002). Also, furfural synthesis occurs in the heated pot (Balcerek et al., 2017), mainly during the first distillation (Awad et al., 2017).

At the beginning of the distillation, the distillate had a furfural concentration of 0.69±0.39 mg/100 mL a.a. (Figure 3a). However, during the middle and end of the first distillation, an increase in furfural concentration was observed, reaching values of 3.81±0.61 mg/100 mL a.a. for the last sample (Figure 3a). That is, when the ethanol concentration decreased, the furfural concentration increased, since furfural is very soluble in water and has a high boiling point (Zhao et al., 2014); therefore, this furan is predominant in the tail fraction.
behavior of the regulated chemical compounds to establish suitable cuts of the distillate. Results suggest that the measurement of ethanol concentration during the distillation process can be helpful to define the distillation cuts adequately and improve the chemical and organoleptic qualities, and the Maestros mezcaleros can certify their Mezcal.

ACKNOWLEDGEMENTS

The authors thank the distillery “Los danzantes” for allowing us to collect the distillate samples during the artisanal distillation process.

REFERENCES


