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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

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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), 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. (alcohol anhidro) de ésteres; 421.21, 452.28 y 40.26 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

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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, mezcals 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 cocuy de pecaya (agave spirits), these volatile compounds also have the same behavior as in the spirits mentioned above (Prado-Ramírez *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 a100 mL sample for each liter of distillate. Samples were collected in 100 mL plastic bottles and stored at 4ºC 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 4ºC 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

4100M, Switzerland). One milliliter of the sample was injected into the digital densitometer, and the values were recorded. Samples were analyzed in triplicate.

Volatile compounds determination

The compounds regulated by NOM-070-SCFI-2016 were analyzed following the methodology described in the Mexican standards NMX-V-005-NORMEX-2013 (aldehydes, esters, methanol, and higher alcohols) and NMX-V-004-NORMEX-2013 (furfural).

The concentrations of methanol, higher alcohols (1-pentanol, isoamyl alcohol, n-butanol, isobutyl alcohol, 2-butanol, and 1-propanol), esters (ethyl acetate and ethyl lactate), aldehydes (acetaldehyde), and furfural were determined in a gas chromatograph (Shimadzu GC-2010Plus). It was equipped with automatic injection (Shimadzu, ADC-20i), a flame ionization detector (FID), and a CP-WAX 52 CB (50 m x 0.32 mm) column. Injector temperature was 250°C, and detection by FID at a temperature of 450°C. The gas chromatography oven temperature was 250°C. Nitrogen was used as the carrier gas at a 40 mL/min flow rate, air 400 mL/min, and hydrogen 40 mL/min.

Samples were adjusted at 20°C and placed in a 50 mL volumetric flask, then 1 mL of internal standard solution (2-pentanol; Sigma-Aldrich) was added. This solution was placed in 1 mL vials, and one µL of the sample was injected by automatic injection. To identify and quantify volatile compounds, certified standards (Chem Service, Inc., and AccuStandard®) were used, and calibration curves were performed for each compound.

Statistical analysis

During the first distillation, 24 samples of "ordinario" were collected from two stills, and mean values and standard deviations were calculated. The normality of the data for each variable (ethanol, aldehydes, higher alcohols, esters, furfural, and methanol) was analyzed using the Kolmogorov-Smirnov normality test. Continuous quantitative variables were transformed using the expression $((x)+1)^{1/2}$, while the data reported as percentages were transformed using arcsine $(x/100)^{1/2}$ to obtain a better fit in the normality of the data. Then, the behavior of the volatile compounds was compared between stills using a t-test ($p \leq 0.05$) of two independent samples.

On the other hand, one-way ANOVA followed by Fisher LSD test ($p \leq 0.05$) was used to evaluate whether significant differences existed between the concentration of ethyl acetate, ethyl lactate, 2-butanol, n-propanol, isobutyl alcohol, n-butanol, isoamyl alcohol, and amyl alcohol from the samples collected during the first distillation. The data obtained in the second distillation were not statistically analyzed as the number of performed distillations was not sufficient (distillation samples were collected from a single still). The still contained the "ordinarios" previously analyzed. All statistical analysis were performed with the statistical package Minitab Statistical Software V.19.

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 (Balcerek *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, $p \leq 0.05$). 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

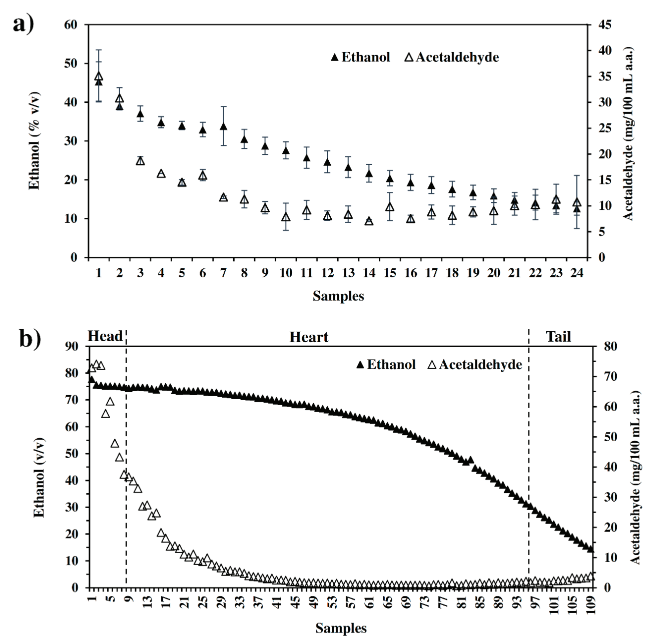

Figure 1. Behavior of ethanol and acetaldehyde during the first (a) and second (b) distillation of artisanal Mezcal. The vertical dotted lines on figure "b" indicate the distillate cuts. In figure "a", concentration values are given as the mean of the samples from two copper stills ± standard deviation.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

experiences. 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, isobutyl 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 575.805±58.85 mg of higher alcohols/100 mL a.a. (Figure 2a), 95 % of them consisted of isobutyl 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 head fraction (Fisher LSD test, $p \leq 0.05$). This is probably due to their low solubility in water (Léauté, 1990) and high affinity to ethanol,

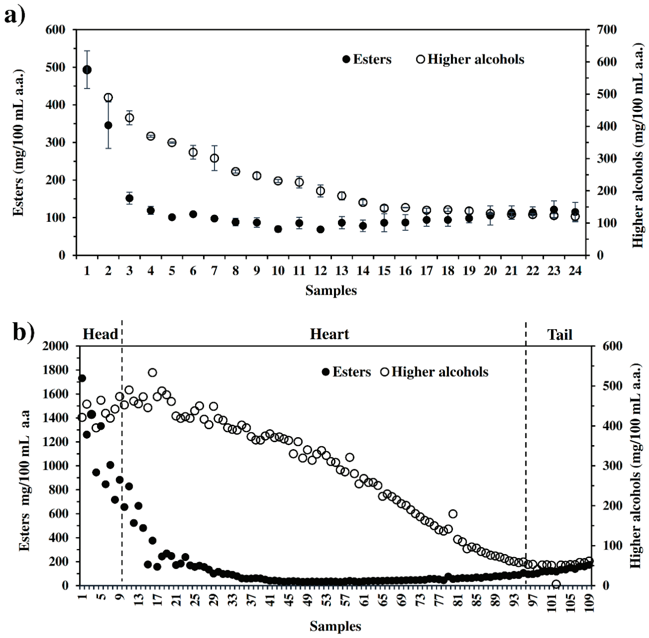

Figure 2. Behavior of higher alcohols and esters during the first (a) and second (b) distillation of artisanal Mezcal. The vertical dotted lines on figure "b" indicate the distillate cuts. In figure "a", concentration values are given as the mean of the samples from two copper stills ± standard deviation.
Figura 2. Comportamiento de alcoholes superiores y ésteres 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.

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).

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.
Tabla 1. Concentración de compuestos volátiles durante la primera destilación del mezcal.

Sample	Compound concentration (mg/100 mL a.a.)							
	Ethyl acetate	Ethyl lactate	2-Butanol	n-propanol	Isobutyl alcohol	n-Butanol	Isoamyl alcohol	Amyl alcohol
1	470±7.59^{a}	21.88±1.7^{8}h	0.88±0.15^{c}	25.21±0.58^{a}	107.06±9.39^{a}	0.60±0.04^{ab}	440.80±49.56^{a}	1.26±0.32^{d}
3	124.09±16.10^{b}	27.82±0.28^{gh}	0.81±0.03^{c}	23.10±2.74^{a}	81.49±5.26^{b}	0.54±0.04^{b}	319.69±16.77^{b}	1.24±0.06^{d}
5	70.26±5.38^{c}	31.26±0.10^{g}	0.83±0.03^{c}	21.88±0.96^{ab}	66.73±1.65^{c}	0.54±0.01^{b}	258.49±4.68^{c}	1.28±0.03^{d}
7	64.95±0.73^{c}	33.03±3.88^{g}	0.88±0.05^{c}	22.12±0.21^{ab}	58.20±6.50^{c}	0.55±0.02^{b}	241.35±32.58^{c}	1.24±0.23^{d}
10	28.49±5.97^{d}	41.42±1.80^{f}	0.82±0.02^{c}	21.15±0.59^{b}	46.36±0.86^{d}	0.52±0.04^{b}	163.67±4.38^{d}	1.43±0.11^{cd}
12	20.47±1.73^{d}	48.50±1.20^{e}	0.89±0.10^{bc}	20.72±0.63^{b}	41.45±1.78^{d}	0.49±0.03^{b}	146.47±16.78^{de}	1.56±0.17^{cd}
15	31.79±19.58^{d}	54.93±4.47^{d}	0.98±0.04^{bc}	20.51±1.13^{b}	30.38±2.10^{e}	0.48±0.09^{b}	89.01±4.58^{ef}	1.56±0.26^{cd}
17	31.06±17.58^{d}	63.36±0.35^{c}	0.97±0.11^{bc}	22.43±1.98^{ab}	29.70±0.11^{e}	0.54±0.05^{b}	87.84±.81^{f}	1.81±0.20^{c}
20	27.97±24.46^{d}	77.91±0.85^{b}	1.06±0.07^{b}	21.79±1.22^{ab}	28.24±0.61^{e}	0.57±0.04^{ab}	78.63±3.91^{f}	2.41±0.01^{b}
24	23.35±20.01^{d}	91.73±5.6^{8}a	1.41±0.10^{a}	21.95±2.81^{ab}	25.70±3.00^{e}	0.77±0.25^{a}	63.14±6.34^{f}	3.28±0.43^{a}

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 $p \leq 0.05$ (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, $p \leq 0.05$), 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 (64.7ºC). 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.
Tabla 2. Concentración de compuestos volátiles durante la segunda destilación del mezcal artesanal.

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

The concentration values correspond to a single measurement of the samples collected from the distillate of the second distillation.

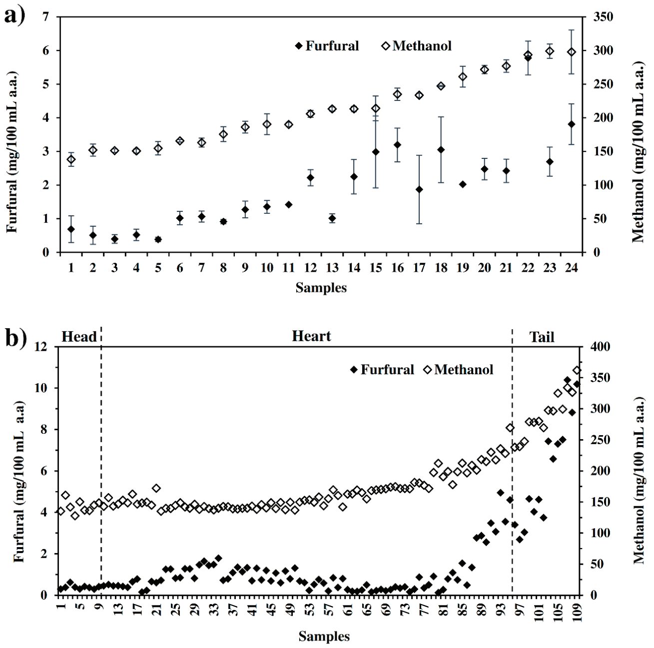

Figure 3. Behavior of methanol and furfural during the first (a) and second (b) distillation of artisanal Mezcal. The vertical dotted lines on figure "b" indicate the distillate cuts. In figure "a", concentration values are given as the mean of the samples from two copper stills ± standard deviation.
Figura 3. Comportamiento del metanol y furfural 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.

Furfural

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). The NOM-070-SCFI-2016 establishes a maximum permissible limit of furfural of 5 mg/100 mL a.a.

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.

In the second distillation, furfural started with a concentration of 0.3 mg/100 mL a.a. This concentration remained constant in the head fraction. Then, its concentration increased from 0.41 to 3 mg/100 mL a.a. approximately in the heart fraction. Finally, while in the tails, it reached concentrations between 3 and 10 mg/100 mL a.a. This data reveals the importance of accurate cuts of the distillate fractions. In this case, if more volume of the tail fraction is collected as part of the heart fraction, the concentration of furfural would increase, and it could even exceed the permissible limit.

According to the results of the present study and the distillation conditions employed, such as the use of butane gas, 300 L copper stills, as well as the maguey species (*A. angustifolia*), it is possible to suggest the distillation cuts during the rectification process as follows: the distillation cut of the head fraction could be performed when the distillate stream has a high ethanol concentration (around 75% v/v); then, the distillation cut of the heart fraction from the tail fraction could be performed when the ethanol concentration in the stream is around 20-30% (v/v); finally, the distillation cut of the tail fraction can be performed at a lower alcohol content (12-14% (v/v) ethanol).

To have greater precision in the distillation cuts, it is recommended to consider the maguey species and maturity stage (Pinal *et al*., 2009), total reducing sugar content, long or short shaving of the stem leaves, environmental temperature, natural fermentation, and region of production, as factors that influence on the synthesis of volatile compounds (Kirchmayr *et al*., 2017; Nolasco-Cancino *et al*., 2019; Ruiz-Terán *et al*., 2019). Also, it is important to draw the distillation curve of each maguey species used in the mezcal production

Although the absolute concentrations of these compounds vary, their behavior and relationship with the ethanol concentration will be the same. That is, when the ethanol concentration in the distillate stream is high (75-77% v/v), higher alcohols, aldehydes, and esters are abundant. Therefore, when there are problems with compliance with the maximum permissible limits of these compounds, the cut of the head fraction will have to be increased (Figure 2b).

On the contrary, methanol and furfural are compounds that predominate when the ethanol concentration in the distillate stream is low (≤20% v/v). Therefore, when there are problems with compliance with the maximum permissible limit of methanol (300 mg/100 mL a.a.) or furfural (5 mg/100 mL a.a.), the cut of the heart fraction from the tail fraction will have to be reduced; that is, this fraction should be separated when the distillate stream has an ethanol concentration >20% (v/v) (Figure 3b).

CONCLUSION

The volatile compounds showed different concentrations among the head, heart, and tail fractions. Esters, higher alcohols, and aldehydes were predominant in the head fraction. While in the tail fraction, methanol and furfural were principal. This study revealed the importance of knowing the behavior of the regulated chemical compounds to establish suitable cuts of the distillate. Results suggest that the measurement of ethanol concentration during the distillation progress can be helpful to define the distillation cuts adequately and improve the chemical and organoleptic qualities, and the Maestros mezcaleros can certify their Mezcal.

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