

Quantification of Mayor Volatile Compounds from Artisanal Agave Distilled: Bacanora

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ABSTRACT

Bacanora is an artisanal beverage distilled from agave, which is manufactured on a small scale in the Sonora state, México. The aim of this study was to identify the major volatile compounds in 77 artisanal Bacanora beverages by gas chromatography, to determine the samples that comply with the Mexican Standards for Bacanora. The samples were collected in 28 municipalities in the area of origin denomination. It was found that only 55.8% of the samples (43) meet the parameters established in the Official Mexican Standards, whereas 44.2% of the samples (34) do not comply: 3 samples for alcohol content, 8 for acetaldehyde, 1 for esters, 11 for methanol and 17 for higher alcohols. Some of the samples do not comply because of more than one analyzed parameter. The Bacanora samples showed great variability among the sampled regions as well as within the same municipalities ($p \leq 0.05$).

Keywords: Alcoholic Beverages; Bacanora; Volatile Compounds; Official Mexican Standards

1. Introduction

Bacanora is a distilled beverage with high alcohol content and is produced on a small scale in the area of origin denomination (AODB), which comprises 35 municipalities of the Sonora state in northwestern México. The traditional production of the drink is performed using wild agave (*Agave angustifolia* Haw). The process starts by cooking the core of the agave, called the “head or pineapple” to hydrolyze inulin, which is the carbohydrate reserve in the “pineapple” into mostly fructose (95%). After grinding and water addition, spontaneous alcoholic fermentation proceeds for 7 to 14 days, depending on the temperature. When the fermentation product is ready, a first distillation is performed. At the end, the bagasse is discarded, and the product is subjected to a second distillation in which three parts are collected: the head, media and tail. Then, the beverage is adjusted or mixed depending on the particular preference of each artisanal producer. This yields a product with an alcohol content

ranging between 38% and 55% [1-3].

Other agave-distilled beverages can be found in México, such as tequila and mezcal, and also there exists the beverage sotol which is manufactured with another plant from the genera *Dasilirium* and processing like tequila Bacanora and mezcal. Each of these beverages had its own area of origin denomination and official Mexican standards [3-5]. Tequila is the only one beverage elaborated an industrial standardized scale and has international recognition. The others are still produced on a small scale and, in some cases, without regulations. Particularly for Bacanora, there are no studies describing the composition of this beverage, in contrast to tequila and mezcal, which are more commercially produced. There exists a report where tequila presented less variability and better complied with the official Mexican standards, followed by mezcal, while sotol and Bacanora had greater variability. However, the quality of an alcoholic beverage is not only measured by its ability to meet the specifications of the official standards which are enforced but also by other factors, such as compounds con-

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tributing to consumer acceptance. The importance of the official Mexican standards is to control beverages which cause damage to humans, described in the Mexican Norm NOM-168-SCFI-2004, such as beverages with high methanol content (**Table 1**) [6].

During alcoholic fermentation performed by yeast, ethanol and CO₂ are the main products of metabolism, but other compounds are also produced which contribute to the flavor (taste and aroma) and are known as secondary products of fermentation [7]. Regarding the composition of tequila, more than 175 volatile compounds have been distinguished, predominating alcohols and low amounts of esters, acetals, terpenes, furans, acids, aldehydes, ketones, phenols and sulfides [8]. In mezcal, an artisanal beverage, 85 different components have been reported, 30 of which have already been reported as important odorants in other alcoholic beverages and 76% of these are found in tequila. The predominating compounds in mezcal were alcohols, esters and organic acids [9,10]. It was also observed that the profile of volatile items depends on the amount and type of the fructans contained in the agave plant [11]. The primary aroma component of these beverages comes from the agave plant; they can undergo chemical transformation or can be modified during the process. The secondary aroma compounds are produced during the cooking, fermentation, distillation and maturity processes [12].

Few studies on Bacanora quality have identified volatiles, such as ethanol, organic acids, esters, aldehydes, ketones, terpenes and other minor hydrocarbons [13-15]. However, there are no studies on whether the beverages currently elaborated in the AODB comply with the parameters established in the official Mexican standards. Currently, efforts are being made to establish formal standard methods of production for industrialization, which has attracted interest in the chemical characterization of the Bacanora produced in the different municipalities of AODB. The aim of this study was to evaluate the major volatile compounds in artisanal Bacanora, which is still produced on a small scale within the AODB.

Table 1. Parameters specified on the Official Mexican Standards for Bacanora [3], for some compounds related with sensorial attributes and safety.

Parameter	NOM-168-SCFI-2004 specification
Plant type	<i>Agave angustifolia</i> Haw
Alcohol strength (alc. vol%)	38 - 55
Aldehydes (g/hL of alcohol)	0 - 40
Esters (g/hL of alcohol)	2 - 200
Methanol (g/hL of alcohol)	30 - 300
Higher alcohols (g/hL of alcohol)	100 - 400

2. Materials and Methods

2.1. Samples

A total of 77 samples were obtained of the beverage called Bacanora produced in the different municipalities of AODB. This area was divided into 4 regions: 18 samples from the Sonora River (7 municipalities), 20 samples from the High Sierra (10 municipalities), 21 samples from the Sierra Baja (10 municipalities), and 16 samples from the Central and Southern Region (9 municipalities) [3].

2.2. Analysis of Alcohol Content

The alcohol content, expressed as % alcohol volume (% alc. vol.), was evaluated using a set of calibrated breathalyzers (Dujardin-Salleron, Paris, France) and a calibrated thermometer (Kessler, USA) [16].

Chromatographic Analysis

The quantitative determination of the major volatile compounds acetaldehyde, ethyl acetate, acetal, methanol, 2-butanol, ethyl butyrate, 1-propanol, 2-methyl-1-propanol, 2-propen-1-ol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol was performed using gas chromatography (GS) with flame ionization detection (FID). The method was based on the norm published by the Mexican government [3]. The GS system used was a Varian CP-3800 model. The separation was performed using a capillary column (US5247141H DB-WAX J. W. Scientific) with a 60 m × 0.25 mm internal diameter and a film thickness of 0.25 µm. The carrier gas was nitrogen, set at a flow rate of 1 mL/min. Nitrogen was also used as a make-up gas, or auxiliary, set at a rate flow of 25 mL/min, with hydrogen and air set at flow rates of 30 and 300 mL/min, respectively, at the detector. The temperature program used consisted of an initial hold at 34°C for 12.5 minutes, followed by a gradual warming of 4°C/min to 105°C and then a second gradual warming of 1°C/min to reach a final temperature of 150°C, which was maintained for 1 minute. The injection port temperature was set at 200°C. The samples were directly injected after adding the internal standard (2-pentanol) using a split injection mode in an automated manner (1 µL, 10:1). For the quantification, peak area ratios of different volatiles according to the internal standard were calculated as a function of the concentrations of the substances. The programming of the gas chromatograph oven temperature and carrier gas flow was selected to achieve greater clarity and definition of the separation of the peaks in the chromatograms without interference by the overlap of peaks.

2.3. Statistical Analysis

All data were processed using the statistical package

software NCSS 2007 (Kaysville, Utah, USA). Initially, descriptive statistics were used to understand the centrality and dispersion of the data. Subsequently, one way analysis of variance (ANOVA) was performed to estimate differences between regions. Mean comparisons were performed with the Tukey-Kramer test. Significance was estimated at a 0.05 probability level with a type I error.

3. Results and Discussions

The samples were collected from the 28 municipalities considered within the AODB. Bacanora beverages analyzed in the present work were elaborated with 100% *Agave angustifolia* Haw. It was observed at the time of sampling that some producers elaborate a beverage called “Bacanora”, but they do not use the “pinapple” from *Agave angustifolia* Haw, as the raw material, as stated in the Mexican Official Standards [3]. Instead, these beverages are produced with *Agave lechuguilla*, which is abundant in the High Sierra, but these beverages were not included in this study.

3.1. Alcoholic Content

Most of the analyzed samples, except four, were in agreement with the Mexican official standards for alcohol content [3]. Three of them exceeded the 55% alc. vol. standard for alcoholic strength, and one of them showed an alcoholic content below the lower level of the official standard of 36.7% alc. vol. **Table 1** shows the regulated parameters and the established content in chemical composition for safety described in the official Mexican standards for Bacanora. Ethanol is one of the main products of yeast fermentation. However, some higher alcohols may be produced by yeast during the catabolism of amino acids or from the reduction of aldehydes. Esters are also produced by esterification reactions of ethanol and other alcohols [8-10]. The excessive production of acetaldehyde and ethyl acetate is often correlated with hygiene practice, considering that the fermentation used in this kind of production is natural; not only yeast are involved, other microorganism are present, such as spoiler bacteria [15].

3.2. Chromatographic Analysis

The results of the analysis by gas chromatography showed that 39% of the samples did not meet the requirements established in the official Mexican standards. Six samples (8%) exceeded the parameters for aldehydes (as acetaldehyde), with a concentration higher than 40 mg/dL (or g/hL of alcohol). One sample (1.3%) exceeded the parameters for esters (concentration of ethyl acetate and ethyl butyrate), with concentrations greater than 200 mg/dL of anhydrous alcohol. Eleven samples (14.6%)

showed methanol concentrations over 300 mg/dL of anhydrous alcohol. Seventeen samples (22.6%) showed higher alcohol (*i.e.*, the sum of 2-butanol, 1-propanol, 2-methyl-1-propanol, 2-propen-1-ol, 1-butanol and 2/3-metil-1-butanol) concentrations greater than 400 mg/dL of anhydrous alcohol. **Table 2** shows descriptive statistics of the results of the samples in the different regions of AODB. There was great variability in the volatile content among the different regions and even among samples of the same region. The Sonora River region shows the highest variability between samples, with higher coefficients of variation than the other regions. It was observed that the Sierra Baja region had the highest mean of five compounds specified in the Official Mexican Standards, including acetaldehyde and methanol. This high variability is attributed to the process because Bacanora is still elaborated in an artisanal way, which produces unique, irreproducible features for this spirit beverage, even by the same producer. **Table 3** shows the results of the ANOVA of the groups of compounds that are specified in the Official Standards for Bacanora. It was observed that the ethanol, aldehydes and methanol contents were significantly different between regions ($P \leq 0.05$). The Sierra Baja region showed the highest means in ethanol, aldehydes and methanol and was significantly different from the values observed in the central/southern regions. No significant differences were found in higher alcohols and esters among the regions sampled.

The higher alcohols described in this study are those known as fusel alcohols or fusel oils (alcohols with two or more carbons) formed by fermentation. Excessive concentrations of these fractions may cause undesirable flavors, sometimes described as spicy, hot or solvent-like. During distillation, fusel alcohols are concentrated at the end of the process (known as the tail among Bacanora producers). The higher alcohols have an oily consistency, which is noticeable in distilled products, hence, the name fusel oil [17]. It was observed that these groups of compounds were the most problematic in this study, when they were compared with the parameters described in the official standards. These compounds showed the largest numbers of samples with concentrations higher than those specified in the official Mexican standard (22.6% of the samples). The distillation method used for bacanora production is simple distillation in metallic tank “alambique”, and the last part of the distilled product (known as the tail or colas) is used to dilute the middle fraction of Bacanora. Few producers use distilled water to dilute the middle portion, which is between 60% and 70% alcohol, to create the final alcohol content of the beverage, according to the Mexican NOM.

These practices create a higher alcohol content than what is outlined in the Official Mexican Standards.

Table 2. Described statistic of the quantification of the major compounds in the Bacanora obtained from AODB by regions.

Region/Compounds	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Central and Southern													
Media	44.31	8.37	37.46	6.57	152.29	0.013	0	17.58	64.43	0.412	0.602	183.76	0.233
S. D.	1.85	5.66	41.61	4.36	63.84	0.052	0	5.39	32.76	0.262	0.276	60.86	0.458
C. V.	4.17	67.62	111.07	66.36	41.92	401.53	0	30.65	50.84	63.70	45.84	33.11	196.19
Min.	41.3	2.22	8.66	1.84	81.3	0	0	10.37	36.81	0	0.36	97.73	0
Max.	47.6	18.8	153.68	16.22	269.67	0.202	0	28.75	163.18	0.8562	1.43	263.97	1.72
River Sonora													
Media	45.34	16.37	70.9	15.90	228.58	0.121	0.175	22.80	50.59	0.598	0.617	148.75	0.527
S. D.	5.57	23.09	57.55	28.18	112.47	0.165	0.499	11.24	27.34	0.834	0.417	55.06	0.764
C. V.	12.28	141.03	81.17	177.23	49.20	136.27	284.34	49.29	54.04	139.56	66.67	37.01	144.9
Min.	36.7	0	8.57	2.86	83.69	0	0	9.91	27.8	0	0.283	67.52	0
Max.	55.7	108.76	192.86	128.88	580.51	0.477	2.18	50.56	139.31	3.98	2.1	318.3	2.79
High Sierra													
Media	47.26	10.43	35.64	9.31	145.25	0.441	0.390	20.17	35.88	0.556	1.001	138.15	0.595
S. D.	3.39	7.18	21.82	6.48	47.13	0.565	0.537	13.70	17.10	0.537	0.865	68.39	0.819
C. V.	7.17	68.83	61.22	69.6	280.91	128.23	137.50	67.92	47.65	96.62	86.48	49.50	137.53
Min.	41.8	1.79	7.51	1.24	110.23	0	0	7.07	4.17	0	0.322	13.13	0
Max.	52.8	24.03	82.79	22.29	573.84	2.48	1.91	70.15	61.33	2.35	4.09	239.98	2.739
Sierra													
Media	47.27	30.96	69.14	35.07	280.91	0.211	0.262	24.61	47.61	0.5598	1.596	156.31	0.546
S. D.	3.39	34.21	103.10	46.93	131.82	0.416	0.515	10.76	16.54	0.292	1.57	44.24	1.28
C. V.	7.5	110.49	149.11	133.81	46.92	197.20	196.08	43.72	34.74	52.30	98.74	28.30	2.3
Min.	41.9	3.94	10.94	3.14	110.23	0	0	11.09	19	0.263	0.313	76.8	0
Max.	56.8	151.02	490.51	213.44	575.84	1.78	2.23	62.45	76.97	1.494	7.13	240	5.73

S. D.: standard deviation; C. V.: coefficient of variation; Min.: minimum value in the samples; Max.: maximum value in the samples; I, ethanol; II, acetaldehyde; III, ethyl acetate; IV, acetal; V, methanol; VI, 2-butanol; VII, ethyl butyrate; VIII, 1-propanol; IX, 2-methyl-1-propanol; X, 2-propen-1-ol; XI, 1-butanol; XII, 2-methyl-1-butanol and XIII, 3-methyl-1-butanol.

Table 3. Analysis of variance of the compounds grouped according specified on the Official Mexican Standards (NOM-168-SCFI-2004).

Compounds	Regions					
	Central/Southern	River Sonora	High Sierra	Sierra	ESM	P value
Ethanol	44.31 ^a	45.34 ^{ab}	47.26 ^{ab}	48.42 ^b	0.88	0.01*
Aldehydes	3.37 ^a	11.50 ^a	10.43 ^a	30.96 ^b	4.93	0.002*
Methanol	152.29 ^a	228.58 ^a	145.25 ^a	280.91 ^b	21.84	0.001*
Esters	37.46 ^a	71.08 ^a	36.36 ^a	69.40 ^a	16.93	0.09
Higher alcohols	389.04 ^a	344.03 ^a	311.50 ^a	343.49 ^a	21.35	0.06

ESM: error standard of the media; *Significant differences $P \leq 0.05$.

However, methanol is produced during the cooking process of agave “pinepples” due to the demethylation of pectins in unripe agaves or by high temperatures during cooking and the low pH of the agave juices ready for fermentation (“saite”). Moreover, there are published data which refer to the production of methanol by some strains of yeast that have the enzyme pectin methyl trans-

ferase, which hydrolyzes agave pectin [7,18,19]. Methanol is a compound that, in high concentrations, may cause damage to humans, like blindness. The mean concentrations of methanol for all analyzed samples were relatively low, as in other Mexican beverages, such as tequila and mezcal. In this study, the highest concentration was 580 mg/dL of anhydrous alcohol, but according

to Lachenmeier *et al.* (2006), these levels are not yet of toxicological relevance [3,15], although the Official Mexican Standards require a concentration of 300 mg/dL or less.

Lachenmeier *et al.* (2006) reported that Bacanora was characterized by high concentrations of acetaldehyde; however, in our analysis, only 8% of the samples showed high concentrations of acetaldehyde. They also evaluated only 13 samples of Bacanora, and of these, six (46%) contained high concentrations of methanol, with the highest concentration of anhydrous alcohol at 601 mg/dL. Their results in quantification are similar to the results on this study [15].

The main volatiles for tequila and mezcal agave are higher alcohols, esters, aldehydes and methanol, the most abundant higher alcohols being amyl alcohol, isoamyl, isobutanol, n-propanol, n-butanol and 2-phenylethanol. Yeast has been implicated as the most important influential factor in the formation of volatiles. Native strains in tequila have been reported to be producers of a large quantities of compounds, such as isoamyl alcohol (2-methyl-1-butanol) and isobutanol (2-butanol), compared to strains used in baking [10,12].

According to the results obtained from Bacanora, these volatile compounds are also present in high quantities; however, in tequila, they meet the specifications of the Official Standards. Based on this fact, we can deduce that the native strains present in the preparation of Bacanora have characteristics similar to the native strains present in the production of tequila.

The Bacanora industry is still developing, and there is a need for a regulatory council or official institution to regulate this beverage, as exists for tequila and other beverages. Interest in Bacanora studies has been generated by its producers to assess their products to standardize the process and to offer a better product [1,20]. Given the lack of studies on Bacanora, especially on the compounds that characterize this beverage, we recommend that studies be performed to evaluate both the chemical and the sensory composition (including minority compounds), as has been carried out for tequila and mezcal. However, producers also believe that it is important to maintain the identity of their Bacanora and retain their organoleptic characteristics, which can be accomplished by conducting this type of study.

4. Conclusion

In this study, it was demonstrated that the Bacanora beverage samples showed great variability, and only 55.8% of them meet the parameters established in the Official Mexican Standards. As Bacanora industry is still developing, it is important to standardize the process to produce the beverage, as it has been carried out for other Mexican spirituous beverages. Also it is important to

maintain the identity of Bacanora and retain their organoleptic characteristics which will make the differences with other fermented agave beverages.

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