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Identification of ecotypes of *Agave maximiliana* Baker and their differences in biomass production and total reducing sugars

Identificación de ecotipos de *Agave maximiliana* Baker y su diferencia en la producción de biomasa y azúcares reductores totales

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ABSTRACT

Raicilla is a distilled beverage made from the fermented juice of Agave maximiliana pineapples, which grow wild in the Sierra Occidental of the Jalisco state. This study aimed to identify the different ecotypes of Agave maximiliana present in the Sierra Occidental of the Jalisco state and distinguish their biomass production and reducing sugar content. This work was carried out in 2020 in Mascota, Jalisco, located 195 km west of Guadalajara, Mexico. Biomass production was measured for each identified ecotype by collecting samples from four plants, aged between 2 and 8 years. Each agave pineapple was weighed, with each weight representing one replicate. Reducing sugars were quantified using the DNS (3,5-dinitrosalicylic acid) technique. Seven variants were identified, with no significant statistical difference in biomass between them in the first three years. By eight years, the average yield ranged from 14.25 kg to 47.87 kg, depending on the ecotype. Reducing sugar production ranged from 32 % to 83 %. The ecotypes identified as Typical and Ribbed were the best regarding biomass production and total reducing sugars.

KEY WORDS: *Agave maximiliana*, Biomass, Ecotypes, Reducing sugars.

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RESUMEN

La raicilla es un destilado, producto del fermento de jugos de piñas de *Agave maximiliana*, que se encuentras en forma silvestre en las Sierras Occidental del Estado de Jalisco. Por lo que el objetivo de este trabajo consistió en la identificación de los diferentes ecotipos de *Agave maximilina* existente en la sierra Occidental del Estado de Jalisco, con su diferenciación en la producción de biomasa y azúcares reductores. El trabajo se desarrolló en el 2020 en Mascota, Jalisco a 195 km al poniente de Guadalajara, México. Se cuantificó la producción de biomasa de cada ecotipo identificado, llevando a cabo, colectas de cuatro ejemplares, de crecimientos de 2 a 8 años, pesando cada piña, donde cada peso representa una repetición. La cuantificación de los azúcares reductores se realizó por la técnica DNS (ácido 3,5-dinitrosalicílico). Se identificaron 7 variantes, en los cuales la biomasa entre ellos no muestra diferencia estadística los primeros 3 años. A los 8 años el rendimiento promedio varió de 14.25 kg hasta 47.87 kg según el ecotipo. La producción de azúcares reductores mostró rangos de 32 % hasta 83 %. Los mejor ecotipos según su producción de biomasa y azúcares reductores totales fueron los ecotipos denominados Típica y Costillona.

PALABRAS CLAVE: *Agave maximiliana*, Biomasa, Ecotipos, Azúcares reductores.

Introduction

Raicilla is a traditional alcoholic beverage from Mexico, specifically originating in the State of Jalisco. It is produced by distilling the fermented juices of several agave species, mainly *Agave maximiliana*. This beverage is made in 16 municipalities in the State of Jalisco and one in Nayarit, as per the Official Mexican Standard PROY-NOM-257-SE-2021. Like other species of *Agave* spp. used to produce spirits, *Agave maximiliana* produces a high concentration of fructans over time, which are composed of chains of fermentable monosaccharides such as glucose and fructose (Mancilla & López, 2006). However, since the creation of the Raicilla Regulatory Council in 2000 and the achievement of a designation of origin (DO) on June 28, 2019, the importance of establishing the characteristics of *A. maximiliana* has become paramount for raicilla producers who may gain a competitive advantage in the market (López- Santiago *et al.,* 2023). *A. maximiliana* exhibits different ecotypes or variants adapted to various environmental conditions within its distribution area in Mexico. However, there is no specific or fixed number of officially recognized ecotypes, as variability can be quite extensive, and classification may depend on criteria used by botanists and agave specialists. Ecotypes are usually distinguished by characteristics such as leaf shape and color, plant size, and specific adaptations to the climate and soil of different regions. These adaptive variations allow *A. maximiliana* to thrive in a range of ecological conditions, from semi-arid zones to more arid or mountainous areas. In general, *A. maximiliana* is recognized for its remarkable genetic and phenotypic diversity throughout its distribution range, underscoring its ecological importance and potential for conservation and sustainable use (Colunga-GarcíaMarín *et al.,* 2007).

Currently, only five species are recognized for raicilla production: two from the coastal region and three from the mountainous region. Among the latter, *A. maximiliana* is the primary source of raw material, as it is used to produce 90 % of the raicilla in Mexico's mountainous areas, and it is endemic to the country (García-Mendoza, 1995), distributed in Durango, Sinaloa, Nayarit, Jalisco, Zacatecas, and Colima (McVaugh, 1989; González *et al.,* 2009). Until 2003, *A. maximiliana* was classified as a non-timber forest species, with its use and management regulated by the General Law on Sustainable Forest Development, which stated that only 80 % of mature plants could be harvested in the forest, leaving 20 % of the population to flower for conservation purposes. This factor is significant, as raicilla production in the Sierra Occidental has historically relied on collecting mature wild plants. Raicilla producers use only mature plants, indicated by the presence of the floral scape, which, according to Santacruz-Ruvalcaba (2022), appears between 12 and 14 years of age when grown under tree shade, primarily oak (*Quercus*) trees. When the plant is grown in open-field cultivation, the cycle can be reduced to an age of 5 to 7 years. Due to its seed-based reproduction, *A. maximiliana* exhibits high intraspecific variation (Valenzuela & Gaytan, 2012) and, as a result, significant phenotypic variability in plant shapes and sizes.

However, raicilla produced from *A. maximiliana* offers a range of odors and alcohol types that differentiate and identify it, as methanol production in *A. maximiliana* distillates is significantly lower than in those obtained from *A. tequilana* (De León-Rodríguez *et al.,* 2008). Therefore, this study aimed to identify the different ecotypes of *Agave maximiliana* present in the Sierra Occidental of the Jalisco State and to distinguish their biomass production and reducing sugar content.

Material and methods

Study area

The study was conducted in the Mascota municipality, located in the Sierra Occidental Region of the Jalisco state, 195 km from Guadalajara, Mexico. Mascota is situated at 20° 32' N and 104° 48' W. According to the Köppen climate classification, modified by Ruiz- Corral *et al.* (2021), the climate is (A) C (wo)(w) a: a semi-warm, low-humidity climate with summer rainfall, minimal winter precipitation, an average temperature of 21.8 °C, and an altitude of 1,401 masl. The vegetation type is pine-oak forest, with species such as *Pinus oocarpa*, *P. lumholtzii, P. douglasiana, P. devoniana* (McVaugh, 1992), *Quercus excelsa*, *Q. laeta, Q. magnolifolia*, and *Q. obtusata* (González, 1986).

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Identification of ecotypes

Due to its mode of reproduction, *A. maximiliana* exhibits high genetic and phenotypic variability, appearing in the study area as a mixture of various individual types. Thus, it became necessary to distinguish and differentiate the distinct ecotypes of this species based on relevant, stable morphological characteristics, which included:

1. Leaf length, width, consistency, and shape.

2. Shape of the cross-section of the leaf base.

3. Leaf curvature, edge, texture, glaucousness, and color intensity.

4. Shape, color, and uniformity of the lateral spines on the leaves.

5. Shape and length of the terminal spines.

To assign names to each ecotype, open-field interviews in a roundtable format were conducted to define them by consensus, with input from producers, plant gatherers, master raicilla makers (individuals who have learned the craft through generations), and academics.

Botanically, the collected specimens were identified using Gentry's taxonomic keys (1982), and they were corroborated by comparison with specimens housed at the IBUG Herbarium. A copy of each specimen's leaves was deposited in the Herbarium of the Instituto Tecnológico de Tlajomulco, Jalisco (CREG), under registration numbers 9761, 9765, 9766, 9767, 9768, 9774, and 9775.

All samples collected for ecotype identification were from six-year-old plants, as identified by local producers.

Usable biomass

For the study of usable biomass production, the pineapple was considered, which consists of the stem and the basal segments of the leaves or stalk attached to it after trimming or harvest (Figure 1) (Rendon-Salcido, 2007). Four individuals from each identified ecotype, ranging in age from two to eight years, were weighed on a floor scale with a precision of 0.5 g (Ohaus®, USA), with weights reported in kilograms. The harvested plants were randomly selected based on their phenotype.

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Figure 1. Image of the harvestable biomass or “pineapple”, which consists of the stem and leaf base of *Agave maximiliana*.

Reducing sugars

The reducing sugars determination was carried out at the pilot plant of the Instituto Tecnológico de Tlajomulco. Juices were extracted from the stem and basal leaf segments that make up the pineapple using a household juice extractor, accumulating a volume between 200 and 250 mL. This process was performed for each ecotype, from ages two to eight years.

The technique used to determine reducing sugars was based on the 3,5-dinitrosalicylic acid (DNS) method (Bello *et al.,* 2006). The preparation involved the following steps: 5 g of 3,5-dinitrosalicylic acid, 150 g of Na-K tartrate, and 8 g of NaOH were weighed and dissolved in 200 mL of distilled water. The Na-K tartrate was initially added slowly with constant stirring, then distilled water was added to reach a volume of 400 mL, and finally, the 3,5-dinitrosalicylic acid was added slowly. The solution was stirred overnight and brought to a final volume of 500 mL.

The sample preparation for the total reducing sugar determination using the DNS method was as follows:

1 mL of juice was diluted in 100 mL of distilled water, followed by the addition of 5 mL of 50 % hydrochloric acid. This mixture was heated in a water bath at 65 °C for 10 minutes to hydrolyze the sugars, then allowed to cool and reacted with the DNS reagent.

For the calibration curve, glucose solutions were prepared at concentrations of 60, 80, 100, 120, and 140 g/L.

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In 10 mL glass tubes, 0.5 mL of sample and 0.5 mL of the DNS reagent were added. The tubes were placed in a water bath at 100 °C for 5 minutes, then cooled to room temperature, and 5 mL of distilled water was added. The tubes were stirred, and readings were taken using a digital Vis spectrophotometer (Milto Roy, Espectronic 21, USA) at 540 nm, with a blank prepared using the DNS reagent and distilled water.

Total reducing sugar readings were measured in milligrams per gram of raw material and reported as a percentage (w/w).

Statistical analysis

Statistical analyses were performed using the Statgraphics Centurion XVI package, employing a completely randomized design with four replications. The treatments included the identified ecotypes, from ages two to eight years. The study variables were the production of total reducing sugars in the stem and basal leaf segments attached to it, as well as biomass weight. Significant differences were identified through Tukey's analysis of variance, with a confidence level of 95 %.

Results and discussion

Ecotype identification

Figure 2 shows the various ecotypes identified in the Sierra Occidental Region of Jalisco.

Typical Ecotype (Figure 2a): This ecotype is the most frequent in wild populations, comprising over 40 %. Its leaves are straight and semi-erect, with yellow to brown lateral spines and no protrusions or mamelons between them.

Black Teeth Ecotype (Figure 2b): Characterized by a distinct black coloration of both the lateral and apical spines, consistent throughout the plant's life stages.

Ribbed Ecotype (Figure 2c): Features semi-erect, broad leaves with rib-like protrusions along the leaf length, providing additional rigidity despite the width of the leaves.

Folded Tip Ecotype (Figure 2d): The leaves are folded near the tip, forming a channel-like shape and closing towards the distal end.

Notched Ecotype (Figure 2e): Notable for deep, prominent notches between spines along the leaf edges.

Cow Tongue Ecotype (Figure 2f): The leaves display a concave curvature after 50 % of their length, extending toward the tip.

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Drooping Leaves Ecotype (Figure 2g): This ecotype has leaves that lack the rigidity to remain upright, often appearing as if they seek to rest on the ground during development.

In terms of mamelons on leaf edges, all ecotypes displayed them, though they were less evident in some Typical individuals, while the Notched ecotype exhibited the most pronounced protrusions. Leaf color intensity varied, with a gradient from dark green in the Typical ecotype to lighter green in the Notched ecotype.

Cabrera *et al.* (2022) compared various agave variants used in the production of alcoholic beverages and, as in this study, found considerable heterogeneity among the variants employed, particularly when using wild varieties. This observation could provide a basis for defining standardization protocols for raicilla production.

Some distinguishing indicators of the ecotypes are listed in Table 1, showing significant differences in characteristics such as leaf length, width, and spine height among certain ecotypes. For instance, the Black Teeth, Cow Tongue, and Drooping Leaves variants exhibit similarities, with no significant differences in leaf length and width; the latter two also show similar spine heights.

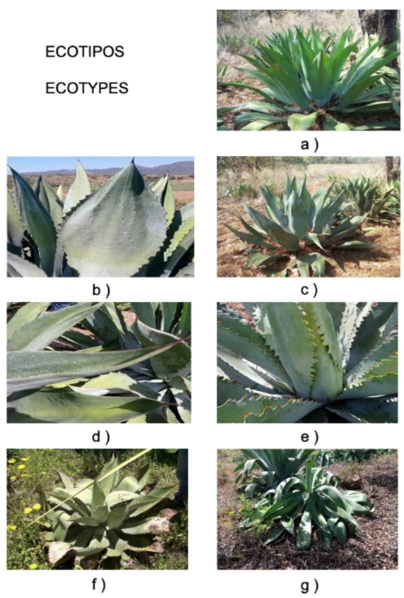

Figure 2. Ecotypes identified in the western highlands of the state of Jalisco, used for the production of raicilla: a) Typical, b) Black Teeth, c) Ribbed, d) Folded Tip, e) Notched, f) Cow Tongue, and g) Drooping Leaves.

Additionally, Table 1 highlights contrasts in the length and width of leaves for the ecotypes evaluated at six years of age. The Folded Tip ecotype had the longest leaves, while the Notched ecotype showed the shortest leaves. The Ribbed ecotype had the greatest leaf width, while the Typical ecotype had the smallest. Regarding spine height, the Notched ecotype displayed the tallest spines, while the Typical had the shortest. Nobel (1987) proposed that leaf width is proportional to changes in leaf length; however, this relationship was not observed in the present study. It is worth noting that Nobel's work focused on different developing agave varieties, and the variability among species may be a reason why this proportionality was not demonstrated here. The data presented in Table 1 are average values from four plants per ecotype.

Biomass Production (pineapple weight)

The pineapple weight from the different ecotypes showed a marked increase starting at five years (Table 2). Although harvesting typically begins at six years and beyond, some plants reach maturity and produce a floral stalk under open-air conditions for raicilla production.

On average, two-year-old pineapples weighed 0.145 kg, and three-year-old pineapples weighed 1.645 kg, indicating an active growth phase; however, no statistically significant difference was found between these two ages.

Table 1. Distinctive characteristics by type of stalk in the seven ecotypes of *Agave maximiliana*

Ecotype	Leaf length (cm)	Leaf width (cm)	Teeth height (with mamelons) (cm)
Typical	107	14.0	0.3
Black Teeth	99	16.5	0.9
Ribbed	98	28.0	0.8
Folded Tip	130	19.6	1.3
Notched	63	17.5	1.5
Cow Tongue	96	19.7	0.6
Drooping Leaves	94	17.5	0.6

The average pineapple weights for the following years were: 2.607 kg at four years, 13.938 kg at five years, 19.571 kg at six years, 27.679 kg at seven years, and 34.125 kg at eight years, with significant statistical differences observed among ecotypes within each age group.

This data is crucial for raicilla production, as it relates to the biomass required to produce one liter of distilled spirit, generally ranging from 10 to 12 kg per liter. If the objective were to

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determine the most desirable ecotype, Folded Tip, Ribbed, and Typical would be preferred for their higher average weights at ages seven and eight. It is important to note that the harvest age for this species is not uniformly based on age but rather on maturity, signaled by the emergence of the floral stalk, which is not uniform across these ecotypes.

Comparing these findings with those of Corbin *et al*. (2016), the average pineapple weight of *A. maximiliana* is lower than that of *A. tequilana*. However, the agave varieties and growth durations differed, which should be considered. Biomass growth depends on various factors, including age, nutrition, biotic and abiotic stress, and variety or ecotypes within the species, as demonstrated in this study. Castillejos-Reyes *et al.* (2023) also found higher biomass in an agave coyote from Oaxaca in an agronomic management study for mezcal production, highlighting that *A. maximiliana* has lower biomass compared to other agaves used for alcoholic beverages.

Tabla 2. Comparison of means based on the average weight (kg) of seven ecotypes of *A. maximiliana* at ages two to eight years.

Ecotypes	2	3	4	5	6	7	8
Typical	0.22 a*	2.03 a	4.13 a	15.69 ab	29.75 a	40.13 b	46.50 a
Black Teeth	0.10 a	1.34 a	2.68 abc	7.13 b	23.50 a	43.1 a	37.50 abc
Ribbed	0.12 a	1.88 a	3.56 abc	9.13 ab	28.75 a	27.00 d	47.88 a
Folded Tip	0.06 a	1.25 a	1.81 c	24.63 ab	30.00 a	21.13 e	42.25 ab
Notched	0.14 a	1.69 a	1.69 c	13.56 ab	11.50 b	26.75 d	30.50 abc
Cow Tongue	0.26 a	1.48 a	1.81 c	11.44 ab	5.75 b	30.25 c	14.25 c
Drooping Leaves	0.15 a	1.86 a	2.56 bc	16.00 ab	7.75 b	12.88 f	20.00 c
LSD value	0.37249	1.0519	2.3354	10.8396	9.8498	20.9046	27.4516

* Different letters show significant difference at α = 95 %.

Reducing sugars quantification

The reducing sugars in pineapples are a crucial indicator for distilled spirit production and biomass production. Together, these components determine the amount of alcohol that can be produced per volume or cultivated surface area. Table 3 shows the content of reducing sugars, which increases as the plant develops over the years. However, a notable decrease is observed in some ecotypes from the seventh to the eighth year, likely due to the delayed removal of the floral stalk, which causes significant sugar loss as the plant uses these sugars for energy. These findings align with results reported by Arrizon *et al.* (2010) and Mellado-Mojica & López (2012), who also observed similar trends in the accumulation of soluble carbohydrates in several *Agave* species.

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Additionally, ecotypes Notched, Ribbed, and Cow Tongue displayed the highest total reducing sugar content, as shown in Table 3. Considering both biomass production and total reducing sugars in pineapples, the ecotypes “Black Teeth,” “Cow Tongue,” and “Ribbed” appear to be the best options for maximizing alcohol yield, followed by “Folded Tip” and “Typical,” with the latter being the earliest maturing variety. Although “Cow Tongue” produces the highest total reducing sugars, it has the lowest potential for biomass production.

Table 4 shows the distribution of reducing sugars within the parts of the agave pineapple, composed of the stem and basal leaves. The basal leaves contain nearly the same proportion of reducing sugars as the stem, explaining why these leaves are also used to produce distilled beverages. However, there is a significant difference in the sugar content of the basal leaves among the different ecotypes.

The average total reducing sugar content in the pineapple of plants aged 6, 7, and 8 years ranged between 32.20 % and 83.07 %. In comparison, *A. tequilana* contains between 27.08 % and 32.69 % from January to May, which aligns with the raicilla harvest season in dry months (Bautista-Justo *et al.,* 2001). Montañez-Soto *et al.* (2011) also reported that *A. tequilana* has a total reducing sugar content of 80.20 %, which is close to the values seen in the “Cow Tongue” and “Black Teeth” ecotypes, which reach 83.07 % and 83.04 %, respectively, as shown in Table 4.

Tabla 3. Total reducing sugar content (%) of pineapple from 2 to 8 years of age of the different ecotypes.

Ecotypes	2 years	3 years	4 years	5 years	6 years	7 years	8 years
Typical	37.35^{a*}	28.60^{d}	37.31^{a}	26.61^{d}	34.16^{b}	40.27^{b}	39.95^{d}
Black Teeth	36.70^{b}	36.14^{a}	36.91^{a}	36.16^{a}	30.86^{c}	37.06^{c}	40.74^{c}
Ribbed	35.98^{c}	36.99^{a}	26.98^{d}	36.99^{a}	31.38^{c}	57.28^{a}	45.39^{b}
Folded Tip	31.14^{d}	31.58^{c}	34.93^{b}	36.70^{a}	36.54^{a}	38.85^{c}	37.03^{e}
Notched	32.87^{d}	21.74^{e}	29.37^{c}	31.37^{c}	34.64^{b}	37.32^{c}	49.08^{a}
Cow Tongue	34.73^{c}	33.96^{b}	35.45^{b}	35.01^{b}	33.72^{b}	34.50^{d}	46.14^{b}
Drooping Leaves	28.20^{e}	36.22^{a}	36.46^{a}	35.29^{b}	36.99^{a}	38.23^{c}	40.34^{c}

* Different letters show significant difference at α = 95 %.

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Tabla 4. Characterization of ecotypes by total reducing sugars from 6 to 8 years in heart and basal leaves.

	Age (years)	T*	BT[1]	R[1]	FT[1]	N[1]	CT[1]	DL[1]
(%) Total reducing sugar on leaves	6	53.75[a**]	37.71[c]	40.83[b]	24.06[e]	41.42[b]	37.08[d]	38.93[c]
	7	24.10[e]	41.20[a]	37.63[b]	40.67[a]	29.17[d]	33.34[c]	37.94[b]
	8	51.66[b]	51.65[b]	66.21[a]	41.95[d]	46.75[c]	41.67[d]	30.18[e]
(%) Total reducing sugars in stalk	6	43.34[c]	53.01[a]	43.89[c]	51.52[b]	42.22[d]	42.24[d]	41.59[e]
	7	45.70[c]	81.34[a]	41.04[d]	37.02[e]	32.20[f]	41.40[d]	57.37[b]
	8	57.61[e]	83.04[b]	79.57[c]	44.28[f]	59.75[d]	83.07[a]	42.65[g]

**T =Typical, BT= Black Teeth, R= Ribbed, FT= Folded Tip, N= Notched, CT= Cow Tongue, DL= Drooping leaves.

* Different letters show significant difference at α = 95 %.

Conclusions

Seven *Agave maximiliana* ecotypes were identified in the Sierra Occidental region of the Jalisco state, labeled in this work as: Typical, Black Teeth, Ribbed, Folded Tip, Notched, Cow Tongue, and Drooping leaves.

Regarding the production of total reducing sugars, the ecotypes Ribbed and Cow Tongue were the ones that presented the highest concentration of reducing sugars. Regarding the production of biomass in the whole pineapple. The "Black Teeth", "Cow Tongue" and "Ribbed" ecotypes could be the best alternatives for obtaining the highest yields of raicilla drink, followed by the "Folded Tip" and "Typical" ecotypes, the latter being the earliest. The "Cow Tongue" ecotype is one of those that produces the most total reducing sugars but has the least potential to produce biomass.

In terms of biomass production, ecotypes with differential growth were observed, covering the range from 14.25 to 47.88 kg per pineapple at the age of 8 years. One point to highlight is that after 6 years of age, the average weight of the pineapples increased markedly, i.e. from years 6 to 7 the weight increased by 19 % and from 6 to 8 years of age the weight increased by 25 %.

Due to how the raicilla is produced, average values should be considered because ecotypes cannot be separated, since this biodiversity should be conceived as a regional richness. The morphological characterization of this work can contribute to protecting this type of agave endemic to Mexico, and thus promote its conservation and sustainable use.

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Author contribution

Work conceptualization; Alfredo Cachúa Torres; Methodology development; Isaac Andrade González, Alejandra Chávez Rodríguez; Alfredo Cachúa Torres; Writing and preparation of the manuscript; Alfredo Cachúa Torres, Isaac Andrade González, I.G. López-Muraira; Drafting, revising and editing.

All authors of this manuscript have read and accepted the published manuscript version.

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Informed consent statement

This statement does not apply to the present work.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Arrizon, J., Morel S., Gschaedler A., & Monsan P. (2010). Comparison of the water-soluble carbohydrate composition and fructan structures of *Agave tequilana* plants of different ages. *Food Chemistry*, 122(1), 123–130. https://doi.org/10.1016/j.foodchem.2010.02.028

Bautista-Justo, M., García-Oropeza, L., Salcedo-Hernández, R., & Parra-Negrete L.A. (2001). Azúcares en agaves (*Agave tequilana* Weber) cultivados en el Estado de Guanajuato*. Acta Universitaria*, 11(1), 33-38. https://doi.org/10.15174/au.2001.325

Bello, D., Carrera, E., & Díaz, Y. (2006). Determinación de azúcares reductores totales en jugos mezclados de caña de azúcar utilizando el método del ácido 3,5 dinitrosalicílico. *Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar*, 40 (2), 45-50. https://

www.redalyc.org/articulo.oa?id=223120664006

Cabrera-Toledo, D., Mendoza-Galindo, E., Larranaga, N., Herrera-Estrella, A., Vásquez-Cruz, M. and Hernández-Hernández, T. (2022). Genomic and Morphological Differentiation of Spirit Producing Agave angustifolia Traditional Landraces Cultivated in Jalisco, Mexico. *Plants*, 11, 1-17. https://doi.org/10.3390/plants11172274

Castillejos-Reyes, C., Bautista-Cruz, A., Sánchez-Mendoza, S., & Quiñones-Aguilar, E. E. (2023). Response of agave coyote (Agave spp.) to the application of slow-release fertilizers under field conditions. *Revista Bio Ciencias*, 10 e1431. https://doi.org/10.15741/revbio.10.e1431

Colunga-GarcíaMarín, P., Zizumbo-Villarreal, D., & Martínez Torres, J. (2007). Tradiciones en el aprovechamiento de los agaves mexicanos: Una aportación legal y conservación de su diversidad biológica y cultural. *In* En lo ancestral hay futuro: del tequila, los mezcales y otros agaves 229-248 pp. Editores. https://doi.org/10.13140/RG.2.1.5192.1441

Corbin, K.R., Betts N.S., Holst N., Jiranek V., Chambers D., Byrt C.S., Geoffrey B. Fincher G.B., & Burton R.A. (2016). Low-Input Fermentations of *Agave tequilana* Leaf Juice Generate High Returns on Ethanol Yields. *Bioenergy Research,* 9, 1142-1154. https://doi.org/10.1007/s12155-016-9755-x

De León-Rodriguez, A., Escalante-Minakata, P., Jiménez-García, M., Ordóñez-Acevedo, L., Flores, F.J.L., & Barba, R.A.P. (2008). Characterization of Volatile Compounds from Ethnic Agave Alcoholic Beverages by Gas Chromatography-Mass Spectrometry. *Food Technology and Biotechnology*, 46(4), 448-455. https://www.ftb.com.hr/images/pdfarticles/2008/Ocotober-December/46-448.pdf

García-Mendoza, A. (1995). Riqueza y endemismo de la familia Agavaceae en México. *In* E. Linares, P. Dávila, F. Chiang, R. Bye y T. Elias Ed. Conservación de plantas en peligro de extinción: Diferentes enfoques., 51-75 pp. Instituto de Biología UNAM. México DF. https://www.researchgate.net/publication/333982451_Riqueza_y_endemismos_de_la_familia_Agavaceae_en_Mexico_1995

Gentry, H.S. (1982). Agaves of Continental North America. University of Arizona Press.

González, E.M., Galván, V.R. López E.I., Reséndiz, R.L., & González, E.M.S. (2009). Agaves- Magueyes lechuguillas y noas del Estado de Durango y sus alrededores. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional. 163 pp https://www.researchgate.net/publication/322243902_Agaves_-magueyes_lechuguillas_y_noas-_del_Estado_de_Durango_y_sus_alrededores

González, V.L.M. (1986). Contribución al conocimiento del género Quercus (Fagaceae) en el Estado de Jalisco. Colección Flora de Jalisco. Universidad de Guadalajara. 240 pp

Ley general de Desarrollo Forestal Sustentable. Nueva Ley publicada en el Diario oficial de la Federación el 25 de Febrero del 2003. Ultima reforma publicada DOF 24-11-2008. https://www.diputados.gob.mx/LeyesBiblio/pdf/LGDFS.pdf

López-Santiago, M.A., Sánchez-Toledano, B.I., Valdivia-Alcalá, R., Hernández-Ortiz, J., García-Vázquez, R., & Vásquez-Maya, I.I. (2023). Análisis del índice de la ventaja comparativa revelada normalizada para el mezcal, tequila y ron en México, *Revista Bio Ciencias*, 10 e1414. https://doi.org/10.15741/revbio.10.e1414

Mancilla-Margalli, N.A., & López, M.G. (2006). Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasylirion* species. *Journal of Agricultural Food Chemistry,* 54 (20), 7832-7839. http://pubs.acs.org/doi/abs/10.1021/jf060354v

Mc Vaugh, R. (1989). Flora Novo Galiciana. Bromeliaceae to Dioscoriceae. Univeristy of Michigan Herbarium.

Mc Vaugh, R. (1992). Flora Novo Galiciana. Gymnosperms and Pteridophytes. University of Michigan Herbarium.

Mellado-Mojica E., & López M.G. (2012). Fructan metabolism in *Agave tequilana* Weber Blue variety along its developmental cycle in the field. *Journal of Agricultural and Food Chemistry*, 60, 1704– 1713. http://doi.org/10.1021/jf303332n.

Montañez-Soto J., Venegas-González J., Vivar-Vera M., & Ramos-Ramírez E. (2011). Extracción, caracterización y cuantificación de los fructanos contenidos en la cabeza y en las hojas del *Agave tequilana Weber azul. Bioagro*, *23*(3), 199-206. https://www.redalyc.org/articulo.oa?id=85721149007

Nobel P.S., & Valenzuela A.G. (1987). Environmental responses and productivity of the CAM plant, *Agave tequilana*. *Agricultural and Forest Meteorology*, 39(4),319–334. https://doi.org/10.1016/01681923(87)90024-4

Proyecto de Norma Oficial Mexicana PROY-NOM-257-SE-2021, Bebidas alcohólicas Raicilla-Denominación, especificaciones, información comercial y métodos de prueba. https://www.dof.gob.mx/nota_detalle_popup.php?codigo=5650295

Rendon-Salcido, L.A. (2007). Relación entre la edad del henequén (Agave fourcroydes Lem) y la estacionalidad climática anual en la producción de destilado alcohólico. [Tesis de doctorado]. Centro de Investigación Científica de Yucatán. https://cicy.repositorioinstitucional.mx/jspui/handle/1003/573

Ruiz-Corral, J. A., Contreras Rodriguez, S. H., García Romero, G. E., & Villavicencio García, R. (2021). Climas de Jalisco según el sistema Köppen-García con ajuste por vegetación potencial. *Revista Mexicana De Ciencias Agrícolas*, 12(5), 805–821. https://doi.org/10.29312/remexca.v12i5.2988

Santacruz-Ruvalcaba F., Castañeda-Nava J.J., Villanueva-González J.P., García-Sahagún M.L., Portillo L., & Contreras-Pacheco M.L. (2022). Micropropagación de *Agave maximiliana* Baker por proliferación de yemas axilares. *Polibotánica*, 1(54), 139-151. https://doi.org/10.18387/polibotanica.54.9

Valenzuela Z.A.G., & Gaytán, MS. (2012). Sustaining Biological and Cultural Diversity, *Revue d'ethnoécologie,* (2). https://doi.org/10.4000/ethnoecologie.990