Agrociencia

MORPHOLOGICAL AND BIOCHEMICAL ANALYSES OF *Agave salmiana* VARIETIES

Lucila **Márquez-Pallares**[1,2*], Juan **Aguila-Muñoz**[3],
José Amador **Honorato-Salazar**[4], Sergio Rubén **Trejo-Estrada**[1]

[1]Instituto Politécnico Nacional. Centro de Investigación en Biotecnología Aplicada. Carretera Tecuexcomac-Tepetitla km 1.5, Tlaxcala, Tlaxcala, Mexico. C. P. 9070.
[2]Tecnológico Nacional de México. Instituto Tecnológico de Apizaco. Avenida Instituto Tecnológico 418, San Andrés Ahuashuatepec, Tzompactepec, Tlaxcala, Mexico. C. P. 90491.
[3]Universidad Nacional Autónoma de México. Centro de Nanociencias y Nanotecnología. Carretera Tijuana-Ensenada km 107, El Sauzal, Ensenada, Baja California, Mexico. C. P. 22860.
[4]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental San Martinito. Carretera México-Puebla km 56.5, San Martinito, Tlahuapan, Puebla, Mexico. C. P. 74100.
* Author for correspondence: lmarquez@ite.edu.mx

Citation: Márquez-Pallares L, Aguila-Muñoz J, Honorato Salazar JA, Trejo-Estrada S. 2024. Morphological and biochemical analyses of *Agave salmiana* varieties.
Agrociencia. https://doi.org/10.47163/agrociencia.v58i2.2841

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: July 07, 2022.
Approved: February 02, 2024.
Published in Agrociencia:
March 05, 2024.

ABSTRACT

The pulque maguey is a perennial plant that provides several environmental benefits. Although its plantation has been drastically reduced, its primary economic and cultural use is the production of fermented, low-cost beverages. In this study, morphological and chemical analyses were conducted, which contribute to the knowledge that allows for the extraction of high-value products from pulque maguey biomass. The *Agave salmiana* varieties studied were Ayoteco (Ayo), Púa Larga (PL), Manso (Man), Chalqueño (Chal), Blanco Cenizo (BC), and Sha'mini (Sha), as well as a "Verde" variety known as Cosmimaco (VC). The data was analyzed using the Shapiro-Wilk test ($\alpha = 0.05$), followed by multiple means comparison using the Tukey procedure ($\alpha = 0.05$), the Kruskal-Wallis non-parametric test, and the Dunn-Bonferroni test ($\alpha = 0.05$). In order to differentiate the agave varieties by their chemical compositions, a canonical discriminant analysis (CDA) was applied. The lipid percentage (1.1–1.7 %) and the cellulose content (61–71 %) were higher than those reported for other *Agave* species. The BC variety contains a high concentration of cellulose and the least amount of lignin, making it a viable option for the energy industry. The CDA identified two discriminant functions that explained 95 % of the variance between the *A. salmiana* species in this study: ash content and lignin content. The results indicate that the amount of cellulose in pulque maguey leaves is an option to obtain products with a higher commercial value.

Keywords: Agavaceae, canonical discriminant analysis, pulque maguey.

INTRODUCTION

The pulque maguey is a member of the *Agave* genus, which is native to the Americas. Mexico is home to 160 of its 210 species, 119 of which are endemic to the country. There are over 30 reported species of pulque maguey, from which various products can be obtained, such as *aguamiel*, which is fermented to produce pulque (Figueredo-Urbina *et al*., 2021). In this process, the leaves are left as waste on the field, though they can be used in cuisine, as forage, or to produce fiber or bioethanol (Narváez-Suárez *et al*., 2016). The leaves have also been studied for the extraction of secondary metabolites, such as steroidal saponins with pharmacological importance (Sidana *et al*., 2016) and phenols with a wide range of biological applications (Almaraz-Abarca *et al*., 2013).

The most widely used species for the production of pulque are *A. salmiana*, *A. mapisaga*, and *A. atrovirens* (Alfaro-Rojas *et al*., 2007). Depending on the variety, it takes anywhere between 8 and 14 years to produce *aguamiel*. This is why agave crops are disappearing, as they are being replaced by annual plantations such as barley. Furthermore, among other causes are the low cost of pulque and the reduction in its consumption (Ramírez-Manzano *et al*., 2020). Following the productive stage of the maguey, the leaves become agro-industrial waste with a high cellulose content. This biomass could be used in various processes, such as the production of second-generation bioethanol, because it does not compete with the food production areas (Yang *et al*., 2015; Díaz-Blanco *et al*., 2018; Jones *et al*., 2020; Yan *et al*., 2020), as a substrate in the production of fungi (Velázquez-de Lucio *et al*., 2022), and for the production of paper (Jiménez-Muñoz *et al*., 2016) and cellulose nanoparticles (Ponce-Reyes *et al*., 2014).

The name of the pulque magueys varies depending on the area (Ramsay, 2004), so the goal of this study is to characterize the morphology and chemical composition of seven varieties of pulque maguey, thereby contributing to technical knowledge that allows for the development of options for the use of the biomass to produce various commercially viable products, which encourages their plantation.

MATERIALS AND METHODS

Area of study and collection

In November and December 2016, maguey leaves were collected during the aguamiel production stage. In the “San Isidro” ranch, located in the municipality of Nanacamilpa, in the state of Tlaxcala, Mexico (19° 28′ 53′′ N, 98° 33′ 55.2′′ W; altitude 2800 m), the varieties Ayoteco (Ayo), Púa Larga (PL), Manso (Man), and Chalqueño (Chal) were gathered. In the town of Ayotla, located in the municipal area of Zacatlán, Puebla, Mexico (19° 55′ 9.84″ N, 98° 02′ 16.08″ W; altitude 2540 m), Blanco Cenizo (BC) and Verde/Cosmimaco (VC) were gathered. The Sha’mini (Sha) variety was gathered in the town of El Saucillo, in the municipality of Huichapan, Hidalgo, Mexico (20° 19′ 1.4″ N, 99° 42′ 25.2″ W; altitude 2170 m).

At the time of sampling, the morphological measurements of each maguey were taken in triplicate: height, length, and width of the leaves, distance between the teeth, and dimension of the apical spine in mature specimens (aged 8 to 14 years) using a measuring tape (Truper, Mexico). Three leaves (high, medium, and low parts of the plant) were taken from three magueys of each variety. The material was transported in bags, labeled with the relevant data, and refrigerated for later analysis.

Chemical determination

Fragments were cut from various parts of the fresh stalks, and composite mixtures of each variety were prepared in triplicate for humidity determination using a thermobalance (Probacsa, Mexico). The stalks were cut into pieces by variety and the sap extracted with an EX-S industrial juice extractor (International, Mexico). The agave fiber was dried in a Baxter brand oven at 65 °C for 72 h before being transferred to the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) where it was ground in a Thomas-Wiley blade mill (model No. 4, Thomas Scientific, Swedesboro, NJ, USA) and sieved with a T-40 mesh size (420 μm), leaving the material of each variety dry, ground, sieved and homogenized for subsequent determinations. The ash content in the agave samples was determined by weight difference after calcining the plant material for 4 h in a muffle at 500 °C using method 923.03 (AOAC, 2005). Nitrogen levels for each variety were determined using a Kjeldahl DEK-1 micro distiller (Sev-Prendo, Mexico) in accordance with AOAC method 2001.11 (AOAC, 2005). Protein content was estimated using the following equation:

$$Crude\ protein\ (\%) = (\%\ N\ Kjeldahl)\ (F)$$

where % *N Kjeldahl* denotes the percentage of nitrogen determined by *Kjeldahl*, and *F* is the base protein factor of 6.25.

Lipid extraction was carried out in a Soxhlet system with hexane at 60 °C for 6 h. Extracts from agave varieties were eliminated sequentially for lignin determination in accordance with the TAPPI-204 standard (TAPPI, 2007), with an ethanol-benzene mixture (1:2) in the first extraction, ethanol in the second, and distilled water in the third. After the sample was free of extracts, the amount of insoluble lignin was determined using the T222 method, which used 72 % sulfuric acid to hydrolyze and solubilize the carbohydrates in the sample, followed by filtering, drying, and weighing the insoluble lignin. The percentage calculation was done using the following formula:

$$\%\ Lignin = (A)\ 100/W$$

where *A* is the weight of lignin (g) and W is the dry weight of the sample (g).

Statistical analysis

A one-factor analysis of variance (ANOVA) was performed on all variables, considering the general linear model $y_{ij} = \mu + \tau_i + \in_{ij}$, where yij is the variable response, μ is the general mean, τ_i is the effect of the i-th factor, and $\in_{ij}$ is the random error. To ensure the model's residual values were normal, the Shapiro-Wilk test ($\alpha = 0.05$) was performed using SAS version 9.2 (SAS, 2000). A multiple comparison of means was performed using the Tukey parametric procedure ($\alpha = 0.05$) or the non-parametric Kruskal-Wallis test, in conjunction with the Dunn-Bonferroni test ($\alpha = 0.05$) (Corder and Foreman, 2014). The SAS DISCRIM procedure was used to perform a canonical discriminant analysis (CDA) on agave varieties to differentiate them based on their biochemical composition.

RESULTS AND DISCUSSION

The normality test of the residual values using the Shapiro-Wilk method revealed that the probabilities of the value of the statistic (W) for the variables leaf length and width, distance between teeth, and ash content of the agave varieties are significant ($p \leq 0.05$) (Table 1), while the rest of the variables have a value of W greater than 0.05, indicating a normal trend. Most variables measured between magueys differ, except for moisture, proteins, and lipids (F test, $p \geq 0.05$).

Table 1. Results of the Shapiro-Wilk test, F test, and Kruskal-Wallis normality test for the analysis of the morphological and biochemical variables in pulque maguey varieties of the Agave salmiana species.

Variable	Shapiro-Wilk test		F test		Kruskal-Wallis test	
	Statistic (W)	*p* value (Pr < W)	Statistic (F)	*p* value (Pr > F)	Statistic (W)	*p* value (Pr < W)
Height (m)	0.9555	0.4303	24.45	< 0.0001	–	–
Apical spine length (cm)	0.9643	0.6062	5.01	0.0062	–	–
Leaf length (m)	0.9033	0.0001	–	–	39.37	< 0.0001
Leaf width (cm)	0.9589	0.0342		–	29.8	< 0.0001
Distance between teeth (cm)	0.9882	0.0371	–	–	78.61	< 0.0001
Humidity (%)	0.9373	0.1925	1.7	0.1926	–	–
Ash (%)	0.8661	0.0081	—	–	19.08	0.004
Protein (%)	0.9892	0.9963	1.57	0.2276	–	–
Lipids (%)	0.9170	0.0755	2.7	0.059	–	–
Cellulose (%)	0.9476	0.3069	4.89	0.0068	–	–
Lignin (%)	0.9530	0.3868	18.22	< 0.0001	–	–

The F test revealed significant differences ($p \leq 0.05$) in the height of the pulque magueys (Table 1). In a study of 62 varieties of the Salmianae section, Mora-López *et al*. (2011) mention that *A. salmiana* var. *salmiana*, to which Ayo, Man, Chal, PL, BC, and Sha belong, showed the widest morphological variability due to the degree of domestication of the plants, where the height of the maguey and the mechanical protection structures mainly change, so the varieties grown in crops (*magueyales*) are larger. In this study, only PL is grown at a lower height, with no significant difference from BC, the largest of the varieties sampled on the edges of land divisions (boundaries), alongside VC and Sha (Table 2).

Table 2. Comparison of the means of morphological variables among the pulque maguey varieties of the *Agave salmiana* species studied.

Maguey	Height	Length of the AS	Leaf length		Leaf width		Distance between teeth		Description of the AS
	(m)[1]	(cm)	(m)	(Ranges)[2]	(cm)	(Ranges)[2]	(cm)	(Ranges)[2]	
Ayo	4.30 a[†]	6.00 ab	2.36	59.00 a	29.22	28.44 a	4.078	104.85 b	Tg[¶]
BC	2.77 bc	7.47 a	1.47	20.83 c	32.56	47.17 a	4.014	100.89 b	Tg
Chal	2.87 b	5.17 b	1.53	30.72 cb	31.11	39.00 a	6.461	204.32 a	Tr[§]
Man	2.93 b	6.37 ab	1.46	18.94 c	29.44	29.33 a	4.172	106.69 b	Cg[♭]
PL	2.83 bc	6.17 ab	1.64	45.17 ab	31.44	41.17 a	3.806	91.35 b	Cg
Sha	2.13 c	4.17 b	1.57	29.83 cb	23.44	5.33	4.094	103.60 b	Cg
VC	2.13 c	6.00 ab	1.44	19.50 c	29.89	33.56 a	5.586	173.81 a	Cg

Ayo: Ayoteco; BC: Blanco Cenizo; Chal: Chalqueño; Man: Manso; PL: Púa Larga; Sha: Sha'mini; VC: Verde Cosmimaco; AS: apical spine. [1]Tukey test ($\alpha = 0.05$); [2]Dunn-Bonferroni test ($\alpha = 0.05$). [†]Values followed by the same letters are not significantly different to each other. [¶]tubular gray; [§]tubular red; [♭]conical gray.

The apical spine is a structural component of mechanical protection (Figure 1). Magueys with higher levels of domestication have a shorter apical spine (Colunga-Garciamarin and May-Pat, 1997). The BC variety collected on the borders has the largest tubular-shaped apical spine; however, Sha, one of the boundary varieties with the shortest and most conical spine (Table 2), does not meet this characteristic. The Chal maguey's thorn is red, which makes it stand out. The VC variety resembled Chal by having short leaves with the greatest distance between teeth.

In terms of chemical composition, the F test reveals no significant difference ($p > 0.05$) in moisture, protein, and lipid content between magueys (Table 1). Given that the varieties in this study are from the *A. salmiana* species, Ayo is molecularly more related to the *A. mapisaga* species (Trejo *et al*., 2020), as are the "Verde" varieties (Alfaro-Rojas *et al*., 2007). The lipid fraction (1.14–1.63 %) exceeds that of *A. sisalana* (Gutiérrez *et al*., 2008), *A. tequilana*, *A. angustifolia*, and *A. lechuguilla* (0.5–1 %) (Jiménez-Muñoz *et al*., 2016). The lipid fraction contains sterols, fatty acids, fatty alcohols, terpenes, long-

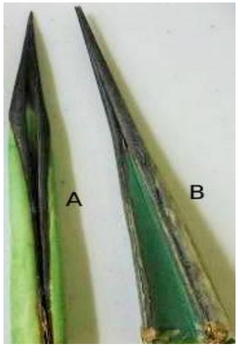

Figure 1. Type of apical spine in the analyzed pulque maguey varieties of the *Agave salmiana* species. A: tubular; B: conical.

chain alcohols, alkanes, ferulic acid esters, hydrocarbon steroids, monoglycerides, aldehydes, and sterol esters.

The cellulose and lignin contents vary significantly ($p \leq 0.05$). The VC variety has the most cellulose (Table 3). In bioenergy research, the most important materials are those with a high cellulose content and a low lignin content. Maguey has been reported to contain 40–80 % cellulose and 16–20 % lignin (Iñiguez-Covarrubias *et al*., 2001; Ponce-Reyes *et al*., 2014). Other studies report lignin percentages ranging from 5.3 to 9.75 % for *A. americana* (Li *et al*., 2014; Jones *et al*., 2020) and 8.4 to 11.9 % for *A. salmiana* (Li *et al*., 2014; de Dios-Naranjo *et al*., 2016). This study's cellulose content is higher than that reported by Yang *et al*. (2015) and Yan *et al*. (2020) for *A. tequilana* (26–26.5 %), and by Díaz-Blanco *et al*. (2018) for *A. lechuguilla* (20.18 %). In contrast, Iñiguez-Covarrubias *et al*. (2001) found 68 % cellulose, similar to the Ayo and VC varieties.

Table 3. Biochemical variables comparison of the Agave salmiana pulque maguey species studied.

Variety	Humidity (%)[1]	Ash		Protein (%)[1]	Lipids (%)[1]	Cellulose (%)[1]	Lignin (%)[1]
		(%)	(Ranges)[2]				
Ayo	87.03 a*	9.18	20.00 a	4.90 a	1.42 a	66.08 ab	16.87 c
BC	87.04 a	4.07	2.00 f	4.30 a	1.14 a	64.50 ab	13.54 d
Chal	85.39 a	5.23	7.33 ed	3.44 a	1.14 a	64.02 b	18.74 cb
Man	86.13 a	5.64	11.00 cd	3.91 a	1.26 a	62.46 b	16.74 c
PL	85.57 a	7.15	15.00 cb	3.97 a	1.37 a	61.02 b	20.05 ab
Sha	83.47 a	7.11	16.00 ab	3.58 a	1.57 a	63.56 b	17.98 cb
VC	85.37 a	4.77	5.67 ef	3.80 a	1.63 a	71.35 a	21.95 a

BC: Blanco Cenizo; Chal: Chalqueño; Man: Manso; PL: Púa Larga; Sha: Sha'mini; VC: Verde Cosmimaco. [1]Tukey test ($\alpha = 0.05$); [2]Dunn-Bonferroni test ($\alpha = 0.05$). *Values followed by the same letters are not significantly different to each other.

The biomass produced by maguey pulque trees can be converted to bioethanol, particularly the BC variety. Although it contains less cellulose, this is compensated by the lower amount of lignin that must be degraded for sugar utilization. Furthermore, in a recent study (Velázquez-de Lucio *et al*., 2022), *A. salmiana* was used as a substrate for the production of the *Pleurotus djamor* fungus with a protein percentage of 4.8 (similar to Ayo), which, despite being treated with urea, was a good alternative due to the chemical composition of maguey bagasse.

The ash content differs between magueys in this study ($p \leq 0.05$). The values of 4.42–9.7 % are similar to those reported for *A. americana* (Jones *et al*., 2020), *A. lechuguilla* (Díaz-Blanco *et al*., 2018), and *A. tequilana* (Yang *et al*., 2015; Yan *et al*., 2020). Chemical composition differences are due to growing conditions, species or variety, age, and analytical procedures used (Jones *et al*., 2020; Yan *et al*., 2020).

The canonical discriminant analysis (CDA) revealed three significant ($p < 0.5$) discriminant functions that accounted for 98.3 % of the variance in agave variety separation (Wilks' Lambda, F = 8.74, $p < 0.0001$, n = 36). The first two discriminant functions describe 95.3 % of the total variation. The FDC1 has the highest eigenvalue (72.85), describing 85.1 % of the variation caused by the ash content; the FDC2 has an eigenvalue of 8.76, expressing 10.2 % of the variation caused by the lignin content; and the FDC3 has 3.1 % of the variation caused by the cellulose content.

The interaction of discriminant functions 1 and 2 (Figure 2) results in the separation of agave varieties into five distinct groups. The PL and Sha varieties are classified as having an ash content of 7 to 7.6 %. Another group consists of the Chal and Man varieties, which vary in ash content from 4.9 to 5.9 %. Ayo, BC, and VC varieties are divided into separate groups. The Ayo variety contains the most ash (9.18 %), whereas BC contains the least ash (4.07 %) and lignin (13.54 %). Finally, the VC variety contains the highest levels of cellulose (71.35 %) and lignin (21.95 %).

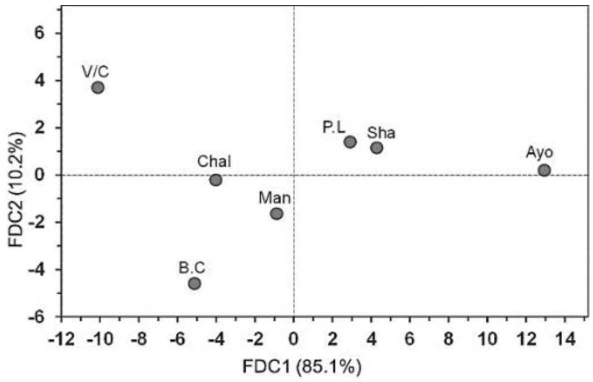

Figure 2. Centroids of two discriminant functions for the analyzed *Agave salmiana* species.

CONCLUSIONS

The BC, Man, and PL pulque maguey varieties share morphological characteristics, including wide leaves and long apical spines. The Ayo variety is the tallest. The canonical discriminant analysis revealed differences in the stalks' chemical composition, particularly in the levels of ash, lignin, and cellulose, the latter two of which are critical in the energy industry. The varieties in this study have a high cellulose percentage (61–71 %), and they can be used to produce bioethanol, particularly the BC variety, which has the lowest lignin percentage and one of the highest cellulose percentages. Although the VC variety is morphologically similar to Chal, the canonical discriminant analysis distinguishes it from the other varieties by containing the highest percentage of lignin and cellulose.

ACKNOWLEDGMENTS

To the *Consejo Nacional de Ciencia y Tecnología* (CONACYT) and the *Tecnológico Nacional de México* (TecNM).

REFERENCES

Alfaro-Rojas G, Legaria-Solano JP, Rodríguez-Pérez JE. 2007. Diversidad genética en poblaciones de agaves pulqueros (*Agave* spp.) del nororiente del Estado de México. Revista Fitotecnia Mexicana 30 (1): 1–12.

Almaraz-Abarca N, Delgado-Alvarado EA, Ávila-Reyes JA, Uribe-Soto JN, González-Valdez LS. 2013. The phenols of the genus *Agave* (Agavaceae). Journal of Biomaterials and Nanobiotechnology 4 (3): 9–16. https://doi.org/10.4236/jbnb.2013.43a002

AOAC (Association of Official Analytical Chemists). 2005. Official methods of analysis. Association of Official Analytical Chemists International. Washington, DC, USA.

Colunga-Garciamarin P, May-Pat F. 1997. Morphological variation of henequen (*Agave fourcroydes*, Agavaceae) germplasm and its wild ancestor (*A. angustifolia*) under uniform growth conditions: diversity and domestication. American Journal of Botany 84 (11): 1449–1465.

Corder GW, Foreman DI. 2014. Nonparametric Statistics: A step-by-step approach (Second edition). John Wiley & Sons: Hoboken, NJ, USA. 288 p.

de Dios-Naranjo C, Alamilla-Beltrán L, Gutiérrez-López GF, Terres-Rojas E, Solorza-Feria J, Romero-Vargas S, Yee-Madeira HT, Flores-Morales A, Mora-Escobedo R. 2016. Isolation and characterization of cellulose obtained from *Agave Salmiana* fibers using two acid-alkali extraction methods. Revista Mexicana de Ciencias Agrícolas 7 (1): 31–43.

Díaz-Blanco DI, de la Cruz JR, López-Linares JC, Morales-Martínez TK, Ruiz E, Ríos-González LJ, Romero I, Castro E. 2018. Optimization of dilute acid pretreatment of *Agave lechuguilla* and ethanol production co-fermentation with *Escherichia coli* MM160. Industrial Crops and Products 114: 154–163. https://doi.org/10.1016/j.indcrop.2018.01.074

Figueredo-Urbina CJ, Álvarez-Ríos GD, García-Montes MA, Octavio-Aguilar P. 2021. Morphological and genetic diversity of traditional varieties of agave in Hidalgo State, Mexico. PLoS ONE 16 (7): e0254376. https://doi.org/10.1371/journal.pone.0254376

Gutiérrez A, Rodríguez IM, del Río JC. 2008. Chemical composition of lipophilic extractives from sisal (*Agave Sisalana*) fibers. Industrial Crops and Products 28 (1): 81–87. https://doi.org/10.1016/j.indcrop.2008.01.008

Iñiguez-Covarrubias G, Díaz-Teres R, Sanjuan-Dueñas R, Anzaldo-Hernández J, Rowell RM. 2001. Utilization of by products from the tequila industry part 2: potential value of Agave *tequilana* Weber *azul* leaves. Bioresource Technology 77 (2): 101–108. https://doi.org/10.1016/S0960-8524(00)00167-X

Jiménez-Muñoz E, Prieto-García F, Prieto-Méndez J, Acevedo-Sandoval OA, Rodríguez-Laguna R. 2016. Caracterización fisicoquímica de cuatro especies de agaves con potencialidad en la obtención de pulpa de celulosa para elaboración de papel. Dyna 83 (197): 232–242. https://doi.org/10.15446/dyna.v83n197.52243

Jones AM, Zhou Y, Held MA, Davis SC. 2020. Tissue composition of *Agave americana* L. yields greater carbohydrates from enzymatic hydrolysis than advanced bioenergy crops. Frontiers in Plant Science 11: 654. https://doi.org/10.3389/fpls.2020.00654

Li H, Pattathil S, Foston MB, Ding SY, Kumar R, Gao X, Mittal A, Yarbrough JM, Himmel ME *et al*. 2014. Agave proves to be alow recalcitrant lignocellulosic feedstock for biofuels production on semi-arid lands. Biotechnology for Biofuels 7: 50. https://doi.org/10.1186/1754-6834-7-50

Mora-López JL, Reyes-Agüero JA, Flores-Flores JL, Peña-Valdivia CB, Aguirre-Rivera JR. 2011. Variación morfológica y humanización de la sección Salmianae del género *Agave*. Agrociencia 45 (4): 465–477.

Narváez-Suárez AU, Martínez-Saldaña T, Jiménez-Velázquez MA. 2016. El cultivo de maguey pulquero: opción para el desarrollo de comunidades rurales del altiplano mexicano. Revista de Geografía Agrícola 56: 33–44.

Ponce-Reyes CE, Chanona-Pérez JJ, Garibay-Flebes V, Palacios-González E, Karamath J, Terrés-Rojas E, Calderón-Domínguez G. 2014. Preparation of cellulose nanoparticles from agave waste and its morphological and structural characterization. Revista Mexicana de Ingeniera Química 13 (3): 897–906.

Ramírez-Manzano SI, Bye R, García-Moya E, Romero-Manzanares A. 2020. Aprovechamiento del maguey pulquero en Nanacamilpa, Tlaxcala, México. Etnobiología 18 (1): 65–76.

Ramsay RM. 2004. El maguey en Gundhó, Valle del Mezquital (Hidalgo, México): variedades, propagación y cambios en su uso. Etnobiología 4 (1): 54–66.

SAS Institute. 2000. SAS/STAT User´s Guide. SAS Institute Inc.: Cary, NC, USA.

Sidana J, Bikram S, Sharma OP. 2016. Saponins of agave: Chemistry and bioactivity. Phytochemistry 130: 22–46. https://doi.org/10.1016/j.phytochem.2016.06.010

TAPPI (Technical Association of the Pulp and Paper Industry). 2007. Acid-insoluble lignin in wood and pulp. T 222 om-02. TAPPI Test Methods. Fibrous Materials and Pulp Testing. Atlanta, GA. USA. 14 p.

Trejo L, Reyes M, Cortés-Toto D, Romano-Grande E, Muñoz-Camacho LL. 2020. Morphological diversity and genetic relationships in pulque production agaves in Tlaxcala, Mexico, by means of unsupervised learning and gene sequencing analysis. Frontiers in Plant Science 11: 524812. https://doi.org/10.3389/fpls.2020.524812

Velázquez-de Lucio BS, Tellez-Jurado A, Hernández-Domínguez EM, Tovar-Jiménez X, Castillo-Ortega LS, Mercado-Flores Y, Álvarez-Cervantes J. 2022. Evaluación del bagazo de *Agave Salmiana* como sustrato para el cultivo de *Pleurotus djamor*. Revista Mexicana de Ingeniería Química 21 (1): 1–14. https://doi.org/10.24275/rmiq/Bio2735

Yan X, Corbin KR, Burton RA, Tan DKY. 2020. Agave: A promising feedstock for biofuels in the water-energy-food environment (WEFE) nexus. Journal of Cleaner Production 261: 121283. https://doi.org/10.1016/j.jclepro.2020.121283

Yang L, Lu M, Carl S, Mayer JA, Cushman JC, Tian E, Lin H. 2015. Biomass characterization of *Agave* and *Opuntia* as potential biofuel feedstocks. Biomass and Bioenergy 76: 43–53. https://doi.org/10.1016/j.biombioe.2015.03.004