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Artisanal refermentation of *Agave cupreata* bagasse with *Saccharomyces cerevisiae* MG5 as a valorization alternative

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ABSTRACT

Objective: to evaluate the economic feasibility of subjecting broad-leaf agave bagasse to secondary artisanal fermentation, with the use of a native yeast to improve ethanol yields and reduce production costs.
Design/Methodology/Approach: the physicochemical characteristics of the bagasse, the amount of sugars according to °Brix, the yields in batches with and without *Saccharomyces cerevisiae* MG5 and the production costs of using the methods described were determined.
Results: the bagasse of *Agave cupreata* contains °6 Brix of total sugar, 4.89 pH, 56.76% organic carbon and total fiber of 16.36%. As for the ethanol yields, those were 15 L Mg^{-1} and 12 L Mg^{-1} with and without added yeasts, respectively.
Limitations/ Implications of the study: a secondary artisanal fermentation process is very expensive and not economically feasible.
Findings/Conclusions: it is important to standardize the processes of use of agave sugars by small producers in order to improve the processes, to increase yields, and to reduce economic losses. For valorization of the bagasse, it is convenient to search for other alternatives.

Keywords: bagasse, broad-leaf agave, fermentation, yields, costs.

INTRODUCTION

Agave, with its high sugar content, is one of the main resources to be used in the distilled beverages industry; approximately 1.5 million tons (Megagrams, Mg) are used. With this activity, solid waste is generated such as the remaining plant bagasse from the

production, approximately 360 thousand Mg per year. Also liquids such as vinasses that end up dumped directly in the lands owned by the producers; these are byproducts with acidic pH and a high content of dissolved organic matter that impacts the environment (Pérez-Zavala *et al.*, 2020; Córdova *et al.*, 2021; Álvarez-Chávez *et al.*, 2021). Agave bagasse is a lignocellulosic waste whose disposal has increased considerably in recent years. It has become an environmental and public health problem that involves economic losses acting as waste (Pérez-Zavala *et al.*, 2020; Cruz-Moreno *et al.*, 2023).

However, despite its great availability and high content of fermentable sugars, as well as the multiple uses and potential applications, the use of bagasse is limited (Montoya-Rosales *et al.*, 2019). The Agave-mezcal production chain is important for the generation of direct jobs and because of the distillate industry demanding for products. As it is already said, there are sugars lost in bagasse; this loss affects especially small producers who refuse to standardize their processes, but remain guided by their ancestral knowledge inherited from generation to generation (Hoz-Zavala & Nava-Diguero, 2017).

Bagasse is a raw material to make various products such as compost, paper, substrate for mushroom production, bio-based construction, biopolymers, surfactants, and biofuels, among others (Kumar & Chandra, 2020; Márquez Aguilar *et al.*, 2022). Unfortunately, not all *mezcaleros* (*i.e.* the people managing Mezcal production) revaluate bagasse. It is important to apply strategies for the use and re-use of mezcal bagasse in order to reduce the environmental impact over mezcal producing areas; particularly those with Mezcal Designation of origin (Hoz-Zavala & Nava-Diguero, 2017; Sierra *et al.*, 2021).

The COMERCAM (Mexican Mezcal Quality Regulatory Council) reported for 2023 a national production of 12 239 655 L of mezcal certified with 45% Alc. Vol. This volume increases year after year due to the international high demand for mezcal. In the production of a liter of mezcal, an average of 12 to 15 kg of agave are used, which depends on the species used. So, it is estimated that for the volume (in liters) produced in 2023, an approximate average of 165 235.34 Mg of Agave was used; and the volume of bagasse generated by this industry is unknown. In addition, the production of non-certified mezcal is not accounted for, and the Mexican Mezcal Quality Regulatory Council (COMERCAM) has no record of it. The state of Guerrero (Mexico) ranked sixth as a national producer of mezcal that year (0.68% of the national production). In this Mexican state, two species of agave (*Agave cupreata* and *Agave angustifolia*) are mainly used. There are no records of how much bagasse is generated in the production of this distilled beverage (COMERCAM, 2024).

This study aimed on a strategy of artisanal re-fermentation of agave bagasse with and without yeasts to evaluate viability in yields and costs of the process. The goal is that small producers who still lose sugars in the bagasse disposal can reduce their losses or improve their production processes at the start.

MATERIALS AND METHODS

Characteristics of the study area

The study was implemented in a site called “Mezcalera La Cueva de la Coyota”, in the Mixtec community El Calvario, located 11 km to the south of Chilpancingo, the capital city of the state of Guerrero (Mexico).

Physicochemical characterization of mezcal bagasse

For the physicochemical characterization of *Agave cupreata* bagasse, 5 kg of fresh bagasse were sent to the Center for Agrifood Innovation and Development of Michoacan (CIDAM). The following parameters were determined in accordance with the corresponding regulations (Table 1).

Pilot test to obtain artisanal alcohol from the re-fermentation of bagasse with and without yeasts

An artisanal production of ethanol was made using 2 Mg of 6 °Brix bagasse in four fermentation tubs, each with 500 kg of bagasse and 100 L of water at a temperature of 28 °C. In two of the tubs, 80 L of water and 20 L of water with 1% sucrose and 10 g of the yeast *Saccharomyces cerevisiae* GM5 isolated from the agave must were added, and were perfectly mixed in both tubs, in order to start the fermentation process. Added yeast was previously characterized at the molecular level; then all tubs were left to ferment for 7 to 9 days. After the elapsed time, distillation was done in an 800 L stainless steel still, and the volume of ethanol was recovered in 20 L jars, following the recommendations of the CIATEJ manual (2014), in order to minimize economic losses in the process. Finally, the volume and alcohol content of both processes, with and without yeast, were measured; so, the yields per Mg of bagasse were determined (Carrasco-López *et al.*, 2024).

Determination of alcohol GL degrees

To determine the alcohol degrees, a Gay Lussac hydrometer was used according to the methodology described by Carrasco-López *et al.* (2024).

Alcohol yield

Alcohol yield is defined as the amount of ethanol produced per amount of feedstock, where RB is the amount of alcohol obtained per gram of biomass, VH (L) is the final volume obtained from each hydrolysate, CR (g L^{-1}) is the concentration of ethanol; and 1000 kg refers to the amount of biomass that was available for each sample (Malagón *et al.*, 2017).

$$RB = \frac{VH * CR}{1000 \text{ kg}}$$

Table 1. Parameters and methodology used for the physicochemical characterization of *Agave cupreata* bagasse.

Parameter	Method
pH, electrical conductivity	NMX-F-317-NORMEX-2013
Total solids, volatile solids, dry and wet weight	NMX-F-428-1982
Total carbohydrates direct calculation with respect to the major components; reducing sugars	NMX-F-312-NORMEX-2016
Total proteins	NMX-F-068-NORMEX-2011
Organic matter content	NMX-F-607-NORMEX-2013
Total fiber (dietary and crude)	NMX-F-613-NORMEX-2017

Cost estimation

Production costs (fixed and variable) were estimated, and a monthly production cycle was assumed. They were compared against the sale prices of ethanol in pharmacies to calculate CRR, the Cycle Rate of Return. In addition, Unit Variable Costs, Unit Fixed Costs and Unit Total Costs were estimated; as well as Profit, for each treatment (Barish & Kaplan, 1978).

RESULTS AND DISCUSSION

The interest in reducing and reusing the bagasse generated in mezcal production arose by initiative of small producers. Various studies have shown the potential for the revaluation of waste from the mezcal industry (Aguirre Fierro *et al.*, 2020). Some producers are aware they lose sugars in bagasse and have considered the possibility of generating alcohol (ethanol) from the discarded sugars that remain in the bagasse of *Agave cupreata*.

Figure 1 shows the refermentation process of bagasse that still contained 6 °Brix after the use of yeasts or without yeasts (Figure 1 A); likewise, distillation after nine days of fermentation is observed (Figure 1 B).

Physicochemical characterization of mezcal bagasse

Table 2 shows the results of the physicochemical characteristics of the bagasse of *Agave cupreata*, in which an acidic 4.89 pH is highlighted, that is very similar to that of species such as *Agave potatorum*, *Agave angustifolia*, and *Agave cantala* (Santiago García *et al.*, 2006). Likewise, *A. cupreata* has 1.20 g in 100 g of direct reducing sugars, equal to *A. angustifolia* (1.4 g in 100 g) and below *A. potatorum* (11.7 g in 100 g). Regarding total reducing sugars and insoluble fibers, *A. cupreata* presented only 2.9% and 9.29% in 100 g respectively, well below other agaves such as *A. potatorum*, *A. angustifolia* and *A. cantala* (170.09, 200.76 and 148.28 g in 100 g; or contents of 13.5%, 14% and 16.7%, respectively) (Santiago García *et al.*, 2006).

All these data are relevant to confirm whether it is feasible to use *A. cupreata* bagasse to produce ethanol, as it has been successfully tested with the residues of *A. angustifolia*, *Agave tequilana* and *A. potatorum*, to mention only a few (Rodríguez *et al.*, 2007).

Figure 1. Procedures for A: refermentation of the *Agave cupreata* with or without *Saccharomyces cerevisiae*; B: distillation after 9 days of secondary fermentation.

Table 2. Physicochemical characterization of *Agave cupreata* bagasse.

Physicochemical parameters	Results
pH	4.89
Electrical conductivity (dS cm^{-1})	3.75
Total solids (%)	17.45
Volatile solids (%)	13.15
Wet weight (%)	84.70
Dry weight (%)	15.30
Total carbohydrates (%)	7.50
Direct reducing sugars (g in 100g)	1.20
Total reducing sugars (g in 100 g)	2.90
Protein (%)	5.38
Total Nitrogen (%)	0.86
Organic Matter (%)	97.85
Total organic carbon (%)	56.76
Ether Extract (total fats) (%)	0.28
Dietary fiber (%) Insoluble fraction (%)	9.29
Dietary fiber (%) Soluble fraction (%)	0.99
Crude fiber (%)	6.08
Total fiber (%)	16.36
Ashes (%)	2.15

Artisanal ethanol production using refermentation of the bagasse of *Agave cupreata*

A volume of 15.5 L (0.0155 g g^{-1}) of ethanol was obtained for each Megagram of bagasse with yeast, and 12.4 L (0.0124 g g^{-1}) without yeasts, both adjusted to 45° Alc. Vol. These yields mean a 20% increase when using *S. cerevisiae* MG5. However, these results are very low compared to those obtained when a single fermentation is done. In those processes volumes of 123.5 0.7 L and 156±1.0 L of mezcal were obtained with and without yeasts respectively. Also, fermentation started at 12 °Brix, and 2 °Brix in the distillate. To the resulting bagasse, Brix degrees [°Brix] were not determined (Carrasco-López *et al*., 2024).

Estimation of ethanol production costs from bagasse re-fermentation

The costs of artisanal ethanol secondary fermentation were estimated. To calculate these costs, the costs of ethyl alcohol were used as a reference. The following indicators were calculated, Profit (which indicates the effective amount obtained from a sale related to the initial investment), and the Cycle Rate of Return (Percentage of the initial investment that returns at the end of each cycle. A negative rate, as it was the case of this work, indicates losses while a positive CRR indicates profits.

Tables below show the Unit Variable (Table 3), Fixed (Table 4) and Total (Table 5) costs that help the producer to know the minimum costs that must be considered in order not to lose their investment. Or else, to redeem the investment in relation to variable expenses (the foreseeable investment in each cycle). In this way, each producer is shown in a simplified way the costs generated by this reprocessing proposal, to validate if the profit they receive is real (Blank *et al.*, 2012; Novelo, 2016).

Results showed that the costs for the artisanal production of ethanol using reprocessed or re-fermented bagasse are higher than expected. Therefore, they are not economically

Table 3. Determination of variable costs of ethanol production for the re-fermentation of agave bagasse with or without yeasts in the bio-manufacture of mezcal.

Variable costs			
Category	**Concept**	**Unit of measure**	**Cost**
Mechanical work	Container filling, weighing y hauling	Working day (15)	$5 520
	Filling of Alembics		
	Distillation		
	firewood cutting		
Technical assistance	Assistance	Honorarium	$1 000
Raw Materials	Bagasse or vinasse	Megagrams	$0
Organics	Yeast	Kilogram	$1 600
Energetics	Firewood	Material transfer (2)	$1 600
	Electricity	kw consumed	$250
Services	Water	Monthly fee	$100
Total $ MXN			$10 070

Table 4. Determination of the fixed costs of ethanol production from the artisanal re-fermentation of agave bagasse.

Costs					
Category	**Concept**	**Unit of measure**	**Useful life (years)**	**Total cost**	**Cost per cycle (monthly)**
Rent	Installations		20	$250 000	$1 041.66
Structures and systems	Hose	Meter	5	$1 000	$16.60
	Tank or cistern	Piece	15	$10 000	$55.55
Machinery and Equipment	Tanks	Piece	10	$7 178	$59.81
	Alembic	Piece	20	$75 000	$312.50
Tools, Accessories and Materials	Carafes (20)	Piece	2	$1 000	$41.66
	Weigh	Piece	20	$3 500	$14.58
	Refractometer	Unit	5	$10 000	$166.66
	Alcoholmeter	Unit	2	$500	$20.83
	shovels	Piece	1	$1 000	$83.30
	Axe	Piece	1	$1 000	$83.30
	Wheelbarrow	Piece	5	$2 000	$33.30
Total $ MXN				$362 178	$1 929.25

*It was considered that approximately 15 L of ethanol are obtained for each Mg of reprocessed bagasse and if a total of 8 Mg per month (8 tubs, 2 per week) is assumed to be processed, then 120 L of ethanol would be obtained for each cycle. A liter of uncertified mezcal (40% Alc. Vol.) sells for an average of $ 80 MXN. With this estimate, up to MXN $ 6400 of net income could be obtained per cycle, which means an income of MXN $ 76 800 per year.

Table 5. Estimation of ethanol production costs in reprocessed bagasse.

Production costs (* L)	$149.99
*Net Profit=IB−CT=6400−11999.25	$5 599.25
Profit margin=(PV−CP)/PV *100	- 87.48%
Rate of return on the cycle=GN/CT	- 46.66%
Total unit costs	$11 999.25
Unit variable costs	$10 070.00
Unit fixed costs	$1 929.25

IB=Net income, CT=Total costs, GN=Profit, PV=Selling price, CP=Production cost (per L); MXN $: Mexican pesos. *It was considered that approximately 10 L of high-grade ethanol is obtained for each Mg of reprocessed bagasse and if a total of 8 Mg per month (8 tubs, 2 per week) is assumed to be processed, then 80 L of ethanol would be obtained for each cycle. A liter of uncertified mezcal (40% Alc. Vol.) is sold for an average of MXN $ 80. With this estimate, up to MXN $ 6 400 net income could be obtained per cycle, which means an income of MXN $ 76 800 per year.

viable to develop is option in a biofactory. This is, mezcal producers should look for alternatives to adjust and standardize their sugar consumption processes in the agave must, so as not to generate economic losses and also, to explore other uses of the bagasse obtained in their facilities.

Some alternatives for the use of bagasse is to hold training workshops to make useful by-products; such as compost, briquettes, animal fodder, adobe bricks, ecological pots, mushroom production substrate, among others. This would expand the possibilities of using this waste to generate additional economical resources for producer families, as well as to reduce environmental impact (Paniagua *et al.*, 2021; Acosta *et al.*, 2023; Ordaz *et al.*, 2019).

CONCLUSIONS

It was shown that it is not feasible to re-ferment bagasse to obtain added value, even if bagasse is discarded with significant amounts of sugars. This is due to re-fermentation costs are high and the extra yields of ethanol are very low. Thus, the recommendation to the producers is to be open to technical training, in order to adjust their mezcal production processes towards consuming all the sugars in the must. Likewise, for the reuse of *Agave cupreata* bagasse, other strategies should be generated and applied to reduce the environmental impact, and to generate additional economic resources for the producer families using broad-leaf agave.

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