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Kinetics and microbiology of pulp silage from decorticated *Agave salmiana* leaves

Cinética y microbiología de la fermentación durante el ensilaje de la pulpa de las hojas desfibradas de *Agave salmiana*

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ABSTRACT

A new machine for decortication of residual leaves of *Agave salmiana* was developed, yielding, juice (43 ± 6 %), wet pulp (25 ± 3 %), and wet fiber (18 ± 4 %) with an overall 86 ± 2 % recovery. Production of lactic acid (LA) and acetic acid (AA) in micro silages of wet agave pulp were assessed together with the evolution of lactic acid bacteria (LAB) and mesophile bacteria (MB) populations. LA spontaneous fermentation was followed by a two stage first order kinetics model (R^2 = 0.89), At the start (pH = 5) MB population was 100 times higher than LAB = 1xE8 CFU/g. Fermentation peak was reached at 11 days when pH = 4, LA = 119 g/kg, LAB = 4xE9 CFU/g, and MB = 1xE8 CFU/g showing that LAB overgrew MB. Average AA values were negligible during the first month. After two months, LA and AA titers were 80 and 40 g/kg, respectively. Pasteurized pulp with LAB < 1E2 CFU/kg showed, significant LAB growth after one week of silage (3xE7 CFU/g). Inoculation of pasteurized pulp by selected LAB strains produced lower counts (8xE9 CFU/g) as compared to spontaneous fermentations (9xE10 CFU/g). Hence, spontaneous agave pulp fermentation is a reliable process for conservation of agave pulp and seems worth scaling-up as the basis for ruminant production in the dry lands of Central Mexico. It remains to develop the utilization of the juice and fiber fractions of *A. salmiana* as the basis for new biochemical and textile industries.

Key words: agave silage, homolactic, kinetics, microbiology, agave leaves

RESUMEN

Se diseñó y construyó una nueva máquina para tallar las hojas residuales de *Agave salmiana* con los siguientes rendimientos: jugo, 43 ± 6 %; pulpa húmeda, 25 ± 3 %; y fibra húmeda, 18 ± 4 %; y una recuperación global de 86 ± 2 %. Se evaluó la producción de ácidos láctico (LA) y acético (AA) junto con la evolución de la población de bacterias ácido-lácticas (LAB) y mesofílicas (MB) en micros silos de la pulpa húmeda. La fermentación espontánea fue seguida (R^2 = 0.89) por un modelo cinético de primer orden en dos etapas. Al principio (pH = 5) la población de MB fue 100 veces mayor que la de LAB = 1E8 CFU/g. El pico de la fermentación se observó a los 11 días, cuando pH = 4, LA = 119 g/kg, LAB = 3xE9 CFU/g, y MB = 1xE8 CFU/g, indicando que la población de LAB predominó sobre MB. Los valores promedio de AA fueron despreciables durante el primer mes de fermentación. Después de dos meses, los títulos de LA y AA fueron: 80 y 40 g/kg, respectivamente. La pulpa pasteurizada mostró cuentas de LAB menores de 1xE2 CFU/g, pero después de una semana hubo un crecimiento significativo de LAB (3xE7 CFU/g). La inoculación de la pulpa pasteurizada con LAB seleccionadas produjo cuentas menores (8xE9 CFU/g) a la fermentación espontánea de la pulpa (9xE10 CFU/g). Por lo tanto, la fermentación espontánea de la pulpa de agave es un proceso confiable para conservar este material como forraje para el ganado rumiante en las zonas semiáridas del Centro de México. Queda por estudiarse y desarrollarse el aprovechamiento de las fracciones líquida y fibrosa de las hojas de *A. salmiana* que podrían ser la base para el desarrollo de nuevas industrias bioquímicas y textiles.

Palabras clave: ensilaje de agave, homoláctico, cinética, microbiología, hojas de agave.

1. INTRODUCTION

In the search to adapt agricultural systems to the global climate change it has been proposed to develop plantations using crassulic acid metabolism (CAM) species because such plants are five times more efficient in the use of water than conventional C3 or C4 plants (Nobel, 1991; Yang *et al*., 2015) and Agavacea is a family of CAM plants that evolved in Mexico and Southwestern USA during the last 10 million years with more than 200 species of *Agave* genus that are well adapted to many local ecological niches of our territory *(*Good-Avila*, et al.,* 2006*).*

Archeological studies in Northern Mexico have shown the ancient use of mixed plantations of agave and maize to overcome famine during droughts (Trombold & Israde-Alcantara, 2005; Anderies *et al*., 2008). Such plantations have survived as, *metepantle,* in small parcels of maguey (*Agave salmiana*), maize (*Zea mays*) and beans (Moreno-Calles *et al.,* 2013). This is presently practiced in more than 10,000 hectares in the Mexican States of Hidalgo, Tlaxcala, Puebla, and Mexico (Ramírez-Rodríguez, 2018).

The main purpose of plantations of *Agave salmiana* and other similar species, is pulque production, a mixed spontaneous fermentation of agave mead (Escalante, *et al.,* 2008). But 80% of agave biomass is wasted or used, after sun drying, as a cheap fuel. Sometimes, fresh agave leaves are chopped using a machete and fed to lambs as a supplementary

feedstuff during the drought (Pinos-Rodríguez, *et al.,* 2008). Manual leaf chopping is a simple practice limited to a few kilograms per day that are insufficient to fulfill the nutritional needs of a small lamb herd or a bovine stable. This problem can be solved by ensiling tons of machine chopped material. Therefore, hand leaf chopping is not sufficient nor efficient to transform tons of agave leaves into a sustainable roughage.

Traditional metepantle parcels are made of mixtures of corn and agave plantations (Moreno-Calles *et al.* 2013). They are composed of 200 to 250 agave plants per hectare (Hernández-Cárdenas *et al.,* 2022) with an average agricultural shift of 10 years and each harvested agave plant yielding 400 kg of fresh leaves. Extrapolation of those figures yield eight tons to ten tons of leaves per hectare and an estimated volume of more than 80,000 tons of leaves in the whole area of pulque production.

Available statistics of tequila industry (Consejo Regulador del Tequila, 2024) show that in 2023 this industry consumed 2.9 million tons of agave stems (piñas). Íñiguez-Covarrubias *et al.* (2001) estimated that leaves of *Agave tequilana* are 29% of whole biomass as compared to 54% of agave stems. Thus, it is possible to estimate around 1.5 million tons of agave leaves usually wasted in tequila fields.

The use of agave leaves as roughage has been reviewed and recommended by García-Herrera *et al*. (2010) and supported by previous work on agave silage by Pinos-Rodríguez *et al.,* (2008) who stressed five basic issues: a) agave leaves have 80% of water content in their parenchyma and become an alternative source of water for lamb production during the drought, b) the digestibility of their organic matter is nearly 60%, c) agave silage can be readily available when conventional roughages are absent or scarce, d) silage fermentation decreased 40% of saponin content of agave and e) agave leaves can be harvested as a by-product of the traditional *pulque* or mezcal production.

Fresh agave leaves can be chopped using a conventional hammer mill, provided with blades to avoid fiber entanglement but, in such a case, fibers are wasted even though they may have more commercial value than agave roughage. Hence it seems interesting to develop an integral and diversified use of such leaves by designing and building a decorticating machine that transforms agave leaves in three fractions: fiber, juice, and pulp.

Agave fibers can be washed out and sundried to be sold as raw material of the textile industry. Leaf juice can be mixed with corn stover and added to the pulp silage. Also, it can be used for industrial production of prebiotic fructo-oligosaccharides (FOS) as suggested by García-Villalba *et al.* (2023) or as a fermentation broth for lactic acid (LA) production that is a raw material for polylactic acid manufacturing (Viniegra-González, 2021). Those alternatives for agave byproducts will not be covered in this paper, yet they support agave leaf decortication instead of the simple use of fresh leaves as a roughage.

Agave pulp, obtained by leaf decortication can be ensiled like chopped agave leaves previously studied by Pinos-Rodríguez *et al.,* (2008). They found it unnecessary to inoculate with starter cultures because such fermentation was homolactic. However, decorticating process increases bacterial contamination of the pulp and seems necessary to study the interaction of wild lactic acid bacteria (LAB) with contaminant mesophile bacteria (MB)

because there is risk of spoilage by MB that often include a broad spectrum of enterobacteria and other undesirable microbial contaminants. It is worth noting that agave pulp is a highly perishable material since it is covered with black molds after one day exposure to environmental conditions and it is not recommended to use it without previous fermentation. Furthermore, it would be interesting to know if pulp silage can be improved by using selected BAL with high fermentation strength. Therefore, it seems useful to study the kinetics of LA and LAB production, compared to the kinetics of MB population and to assess the alternative of pulp pasteurization followed by inoculation of starter cultures.

In this work, a small machine was developed to decorticate *A. salmiana* leaves in three separate fractions, fibers, juice, and pulp. Spontaneous pulp fermentation was followed by the evolution of LA production and the kinetic comparison between LAB and MB populations. Finally, the action of epiphytic (wild) consortia present in agave pulp was compared to pasteurized pulp inoculated with selected LAB. The hypothesis is that wild fermentation of agave pulp is well suited to produce a high and stable level of LA with a low level of remaining MB without the need of expensive pasteurization and starter cultures. This work could be a useful step for the future scale-up of this process at farm level.

2. MATERIALS AND METHODS

2.1. Experimental procedures

2.1.1. Agave materials

Residual agave leaves of *A. salmiana* were purchased from a farm in Coatepec (Iztapaluca, Mex.). They were obtained from mature agave plants that had finished the production of agave mead (*aguamiel*). The leaves were processed in our lab with a decorticating machine specially designed for this work (Fig. 1), having a set of pressing rolling drums adjusted with different spacings and a decorticating drum like one described by Pérez del Río *et al.* (2013).

This new machine could decorticate 50 kg of agave leaves per hour yielding three separate wet fractions: juice, pulp, and fiber.

Fig. 1. Decorticating machine adapted to *A. salmiana* leaves. This machine was provided with a set of adjustable pressing rollers before the scraping drum. Each agave leaf was squeezed by the operator through the pressing rollers to reduce leaf thickness down to 2 cm yielding abundant agave juice. Later, each squeezed leaf was scrapped by a revolving drum provided with transversal blades to separate the fiber from the pulp.

2.1.2. Pulp silage preparation

Fifty milliliter Falcon test tubes were filled with wet agave pulp and covered by a 1 cm layer of finely divided dried pulp. Test tubes were closed with a screw cap sealed with Parafilm to be incubated at 30º C for each fermentation time. For kinetic experiments triplicate samples from different Falcon tubes were sacrificed every day during the first week and every week thereafter, to be analyzed as indicated below. The dry weight (DW) of each sample was determined using a thermobalance (OHAUS). Silage evolution was followed up in terms of product formation (LA) and microbial composition (LAB and MB) normalized by initial DW. Inoculation was not necessary in most samples because the raw material had a strong load of epiphytic (wild) bacteria of unknown nature. The level of initial population of LAB was around 10E8 colony forming units (CFU) per gram of DW.

2.1.3. pH and HPLC analysis

For biochemical analysis, 6 g of wet material were mixed with 50 mL of distilled water and stirred for 10 min, from this sample, pH measurements were obtained. The supernatant samples were processed for HPLC by dilution 1:10 in distilled water and filtered through a Titan 3 nylon filter with 0.45 mm cut-off. High Performance Liquid Chromatography (HPLC)

was done with a Perkin Elmer apparatus having an isocratic Aminex HPX-87h column (Biorad) with a 4 mM H_2SO_4 mobile phase and a steady flow of 0.6 mL/min. Eluate compounds were detected by a refraction sensor (Perkin Elmer). Standard solutions of known compounds (LA, and acetic acid AA) were prepared with a concentration of 1 g/L. For most samples, variation coefficient of triplicates was less than 20%. Standard errors in graphs were not shown when using logarithmic scales for comparison between LAB and Mesophiles CFU data, since they differ in various orders of magnitude.

2.1.4. Microbiological analysis

One wet gram sample of each silage test tube was blended with 9 mL of 0.15% peptone solution on sterile water and vortexed for 5 min. Serial dilutions were made with same solvent up to one billion dilution factors. From each dilution, 5µL samples were evenly spread in a separate sector of a Petri dish and incubated at 37°C.The number of colonies of the highest observable dilution, after 24 h to 48 h, was registered and the average of three replicates, multiplied by the corresponding dilution factor was taken as the number of CFU per gram of DW. Two different culture media were used, MRS and Nutrient Broth.

MRS agar medium (de Mann *et al.,* 1960) is selective for LAB. Mesophile counts (MB) were made on nutrient agar plates (Liofilmchem, 2013). In this culture medium most non fastidious bacteria grow well, including pathogens of genera, *Pseudomonas, Escherichia, Listeria, Salmonella* and *Shigella*.

2.1.5. Inoculation of silage fermentation

Two selected strains of LAB were used, *Enterococcus faecium* ASP.DG8 (OM967272) and *Lacticaseibacillus paracasei* ASA.DG5 (OM802846) previously isolated from an agave plant by Gallardo Martínez *et al.* (2024). Strains were activated from slants in 0.5 mL of MRS broth incubated by one day at 37°C and re-cultured twice in a 1:10 dilution of same broth, for another two consecutive days. Agave pulp was pasteurized for 30 minutes at 90°C and let stand at room temperature to be inoculated with one tenth (v/v) of final silage mash having 10xE8 CFU per g of DW. Control runs were made by pasteurized pulp without inoculation and having a LAB population density lower than 10xE2 CFU per g of DW.

2.2. Kinetic models

2.2.1. Lactic acid production

A two-stage first order kinetic model followed LA production. During the first stage it was assumed that LA was produced following equation (1)

$$LA(t) = (LA_m - LA_0)(1 - e^{-kt}) + LA_0\,;\; 0 < t < t_c \qquad (1)$$

Where, k is the first order kinetic constant; LA_0, is the initial value of LA, and LA_m would be the estimated asymptotic value. After a critical time value, t = t_c, a second stage is defined according to equation (2)

$$LA(t) = (LA_c - LA_r)e^{-r(t-tc)} + LA_r\ ;\ t_c \leq t \qquad (2)$$

Where, r is the first order decay constant; LA_c, is the estimated value of, LA(t), for t = t_c, in equation (1).

Implicit in this model is the assumption that during the first fermentation stage, the limiting factor is the residual concentration of the fermentation substrate. It is assumed that LA production starts to decay when the population of LAB reaches a critical state where excess lactic concentration (LA > LA_c) and low pH, inhibit LA fermentation giving rise to a slow LA decrease until a value, LA_r, is reached. Martha-Lucero *et al.,* (2009) used this model to predict the growth of axenic LAB cultures in well-defined broths supplied with fructans, supplemented by soybean hydrolysates and mineral salts.

2.2.2. Lactic acid bacteria (LAB) evolution

In a similar fashion to LA kinetics, the number, LAB(t), of CFU was assumed to follow two stages. In the first stage it is assumed an exponential model shown in equation (3)

$$LAB(t) = LAB_0 e^{mt};\quad 0 < t < t_g \qquad (3)$$

Where, LAB_0 is the initial population of LAB (t = 0); LAB_g for, t = t_g, corresponds to the number of LAB when the exponential growth finishes, followed by a decay process, given by equation (5), where LAB_f is the asymptotic value of LAB, and, u, is the decay rate constant of LAB.

$$LAB(t) = \left(LAB_g - LAB_f\right)e^{-u(t-tg)} + LAB_f;\ t \geq t_g \qquad (4)$$

2.2.3. Mesophilic bacteria evolution

The number, MB(t), of CFU of MB was assumed to follow two stages, a rapid first order decay model shown in equation (5)

$$MB(t) = MB_0 e^{-vt};\quad 0 < t < t_h \qquad (5)$$

Followed by slow decay shown by equation (6)

$$MB(t) = MB_h e^{-s(t-th)};\quad t \geq t_h \qquad (6)$$

Where, MB_h is MB (t_h) for t = t_h. corresponding to the end point of the fast MB decay.

Estimation of parameters for equations 1-6 was done by minimization of the sum of squared differences, between observed and calculated values, using a Marquardt evolutionary algorithm, embedded in the Solver routine of Excel spread sheet. In each case, the number of data points was more than the double of estimated parameters.

3. RESULTS

3.1. Decortication of agave leaves

Decortication of agave leaves obtained in five experimental runs with the machine shown in Fig. 1, yielded: 43 ± 6%, juice; 24.9 ± 3%, wet pulp; and 17.8 ± 4%, wet fiber. The average leaf weight was 20.2 kg with an overall wet mass recovery of 85% ± 2%. Losses can be explained by juice and pulp retained within the machinery. Dry weight determinations found a similar value of 80% for all samples of wet pulp.

3.2. Evolution of pH through silage fermentation

Figure 2 shows that at the beginning of fermentation pH = 5 and during the first 4 days decayed to values under pH = 4. The pH trend was followed by a moving average. Large variations in pH could be related to heterogeneity of solid samples because it is known that carbohydrate composition is quite different in the apical or basal regions of agave leaves and the sample size was small.

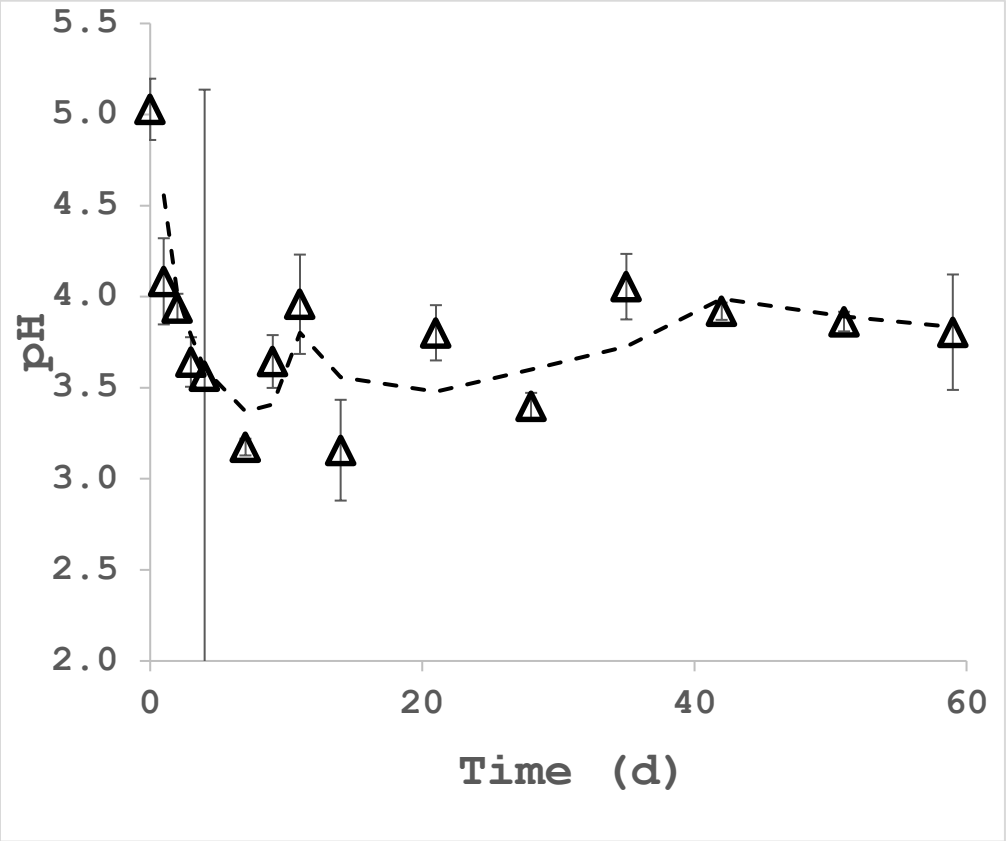

Fig. 2. Evolution of pH measured in aqueous extracts of wet agave pulp silage. Experimental average values of triplicate samples are shown with corresponding standard deviations. Interrupted line is the moving average to smooth pH trajectory. Notice initial pH = 5 and decreasing values to pH lower than four, after three days of fermentation.

3.3. Evolution of lactic acid (LAB) and mesophilic (MB) bacteria in agave pulp spontaneous fermentation

Figure 3 shows the evolution of the number of CFU of LAB and MB per gram of initial DW. The kinetic parameters, corresponding to equations 3 to 6 (Section 2.2.2) are shown in Table 1. At the start MB population was one hundred times higher (MB_0 = 1.4xE10 CFU/g) than LAB population (LAB_0 = 1.4xE8 CFU/g) but at the peak value (t_c = 10.4 days), LAB population (LAB_C = 3.5xE9 CFU/g) overcame MB population (MB_h = 5.6xE8 CFU/g). The first fermentation stage coincides with the initial fast pH decrease shown in Fig. 2. In the same Fig. 3 and Table 1, it is shown that MB decreased extremely fast (v = -4.2/d) during the first few days of silage ($t < t_h$ = 5.8 d) when wild LAB population grew (m = 0.3/d).

After eleven days, both LAB and MB decay at a low pace with values, u = - 0.6/d, s = -0.3/d, respectively, showing the persistence of MB until the end of the observation period.

Table 1. Fermentation parameters of agave pulp silage.

Parameter	g/kg	Parameter	CFU/g	Parameter	CFU/g
t_c (days)	11.0	t_c (days)	10.4	t_h (days)	5.8
LA_m	123	LAB_o	1.4E+8	MB_0	1.4E+10
LA_r	83	LAB_f	1.0E+8	MB_h	5.6E8
r (1/d)	0.3	m (1/d)	0.3	v (1/d)	-4.2
k (1/d)	-0.1	u (1/d)	-0.6	s (1/d)	-0.3
LA_c	119	LAB_c	3.5E+9		
R^2	0.89	R^2	0.97	R^2	0.99

All parameters were estimated using the Marquardt algorithm embedded in Excel spread sheet, using equations of sections 2.2.1 to 2.2.3. Notice that model and data had good correlations ($R^2 \geq 0.9$), as shown in Figs. 3 and 4.

The parameter meanings were:

For LA: t_c, = critical time of LA production; LA_m, extrapolated maximal value; LA_r residual value and, LA_C critical value; r, was the LA production rate, and, k, the LA decay rate.

For LAB: t_c, = critical time of LAB growth; LAB_0, the estimated initial value; LAB_f the extrapolated maximal value; LAB_f, the extrapolated final value; m, was the LAB growth rate; and, u, the LAB decay rate.

For MB: t_h, = transition time from fast to slow MB decay MB_0, the estimated initial value; v, was the fast MB decay rate and, s, the MB slow decay rate.

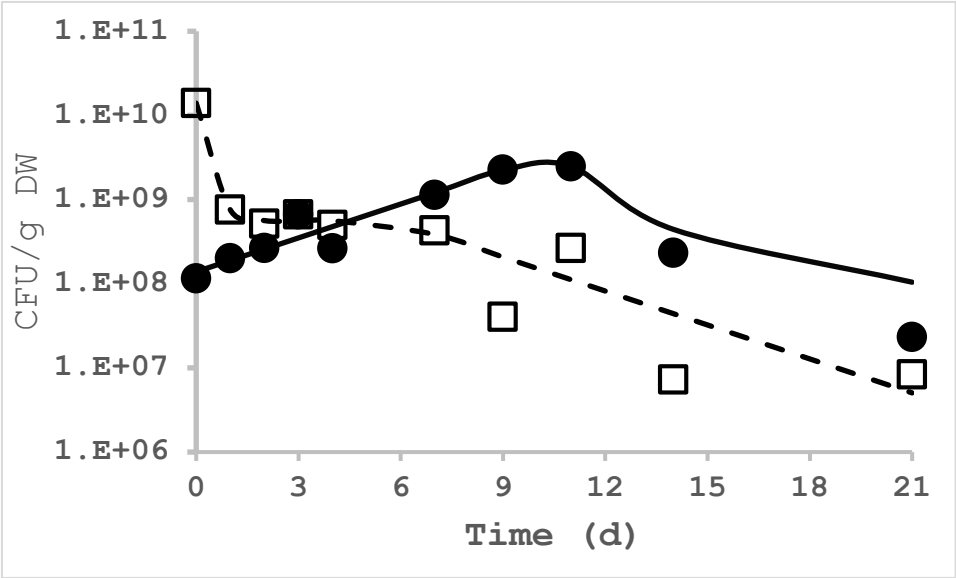

Fig. 3. Evolution of LAB (●) and Mesophile (□) populations during the fermentation of wet agave pulp. Notice the logarithmic scale for CFU expressed in CFU/g of Dry Weight (DW). Humidity was close to 80% in all samples. Both plots had two distinct phases of exponential kinetics, growing, and decreasing for LAB with transition at t = 10.4 days, and two decreasing trends for mesophiles with transition at t = 6 days according to Table 1.

3.4. Kinetics of Lactic Acid (LA) and Acetic Acid (AA) production

Figure 4 and Table 1 show that LA production during the first fermentation stage had a similar production rate (r = 0.3/d) as compared to LAB growth rate (m = 0.3/d). This agrees with a model where lactic acid production is proportional to LAB growth. Figure 4 shows that AA titers were negligible during the first fermentation month, confirming the dominance of lactic acid metabolism over secondary fermentations. But after 30 days, AA titers were significant (40 g/kg). This seems to explain why LA decreased after peak time (LA_C = 119 g/kg) to a final extrapolated value of LA_f = 83 g/kg. The nature and extent of such secondary metabolic reactions remain to be elucidated.

3.5. Effect of pulp pasteurization and inoculation with starter LAB

Figure 5 shows that when the pulp was pasteurized, remaining wild LAB grew to reach a level of 7xE9 CFU/ g. Spontaneous fermentation of agave pulp started at an important level of 9xE7 CFU/g and grew at steady rate to reach 9xE10 CFU/ g like data shown in Fig. 4. Inoculation of pasteurized pulp by selected strains of *Lacticaseibacillus paracasei* or *Enterococcus faecium* did not show significant differences to wild fermentation. Those results support the use of epiphytic (wild) microbiota for wet pulp silage without inoculation with starter strains.

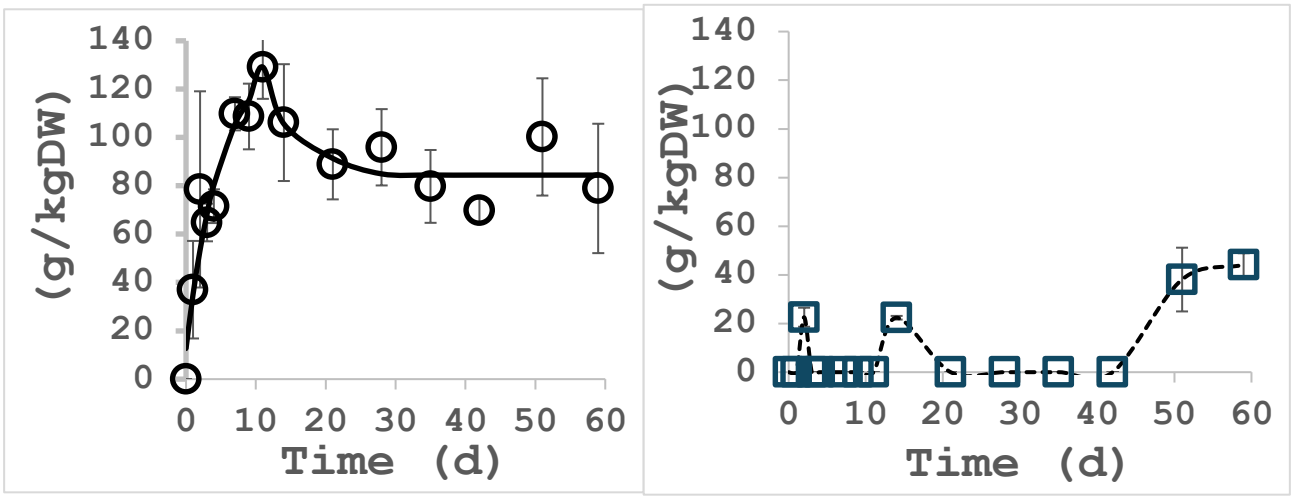

Fig. 4. Spontaneous fermentation of wet agave pulp. (O) Lactic acid titers, (□) Acetic acid titers. Both expressed in g/kg of Dry Weight (DW). Humidity was close to 80% in all samples. Solid line in left graph was traced using equations (1) and (2) of section 2.2.1 with parameters shown in Table 1. Samples of Fig. 3 and 4 were the same for each given fermentation time.

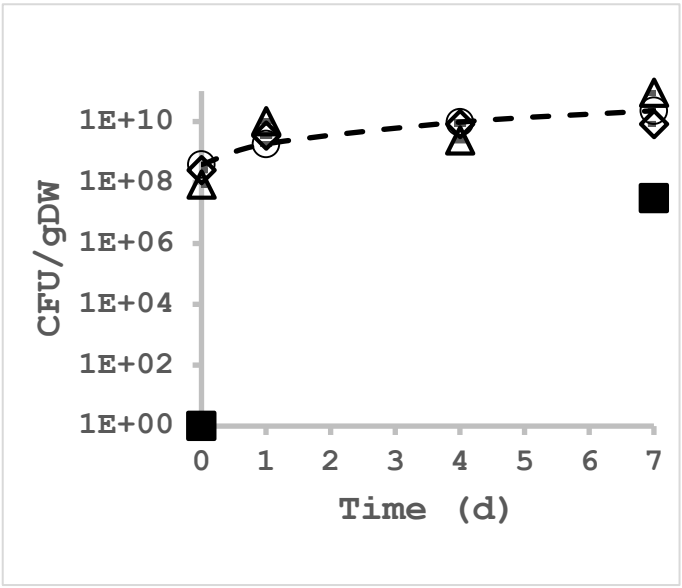

Fig. 5. Microbiological comparison between endogenous and inoculated agave silage. (■) Control, pasteurized pulp, without inoculation. (△) Endogenous (wild) microbiota, without pasteurization, nor inoculation. Pasteurized pulp, inoculated with (O) *Lacticaseibacillus paracasei* or (◇) *Enterococcus faecium.* The initial LAB population of pasteurized pulp was lower than 1xE2 CFU/g. Overall statistical analysis between inoculated and wild fermentations showed no significant differences.

4. DISCUSSION

Present observations show that agave pulp has adequate nutrients and enough epiphytic (wild) lactic bacteria for homolactic fermentation since, in this process, LA was the main fermentation product and LAB population dominated over MB population.

Those observations are related to the notion that there is an optimal carbohydrate to protein ratio, CHO/PROT, favoring lactic acid fermentation over other fermentations such as those producing ethanol or acetic acid. For example, the classical MRS medium, used to screen for LAB, has CHO/PROT = 1:1 (de Mann *et al.*, 1960) whereas, Nutrient Broth composition, used to grow MB, is made of a mixture of beef extract and peptone, without fermentable carbohydrates (Liofilchem, 2013). A long time ago, Viniegra-González & Gómez (1984) proposed that the ecology of mixed fermentations of vegetable material depends on such a ratio. This would explain why cane juice with a CHO/PROT value above one hundred, usually yields ethanolic fermentation, whereas spontaneous milk fermentation with a CHO/PRO value close to one, usually yields high titers of lactic acid. According to published data (Pinos-Rodríguez, *et al*., 2008), agave leaves contain a CHO/PRO = 6. Therefore, present results confirm that agave leaves composition is adequate to produce spontaneous (wild) lactic acid fermentation as required to conserve agave pulp by silage.

During the process of agave leaf decortication there is significant exposure to contaminant microorganisms present in the environment, including MB that may deteriorate the roughage. That is why it was important to show the dominance of LAB over MB. Previous studies (Martha-Lucero, 2019, de Oliveira, *et al.,* 2021) have shown that axenic (pure cultures) of LAB grown in sterilized broths generate a logistic curve of LA production where the final product stays at a maximal value without decay. In this work, LA reaches a maximal value and decays to a lower final value. The coexistence of LAB and MB can explain this phenomenon, where MB use a fraction of LA but, this secondary process is not dominant because the low amounts of secondary fermentation products such as AA found in the silage and the dominance of LAB alongside the fermentation process. In fact, at the start of pulp silage, MB are dominant but their number decays extremely fast during the first week coinciding with the growth of LAB.

There was the question whether pasteurized and inoculated agave pulp would yield better results than those obtained by spontaneous fermentation of this material. Present results did not show significant difference between spontaneous and inoculated fermentation, supporting simple direct fermentation of agave pulp.

Agave leaf mechanical decortication requires a comment as compared to the simpler use of fresh chopped or ensiled agave leaves for ruminant production. The issue is related to economics. Usual feedstuff to meat conversions in ruminants fed with important levels of roughage are in the range of 11 kg of dry mass intake per kg of liveweight gain (Hassan *et al.,* 1990), and agave leaves have 20% of dry mass. Hence, a ton of agave leaves will yield a sales value of 55 USD of lamb (liveweight) at the current price of 3 USD per kg. On the other hand, dry agave fiber yield is close to 40 kg per ton of wet leaves and dry fiber is sold at the current price of 2 USD/kg. Thus, the estimated and combined sales value of fiber and lamb are twice as much as compared to only selling lambs grown on chopped and ensiled agave leaves. Such calculations justify decortication of agave leaves to recover agave fibers and to use agave pulp as roughage.

An apparent drawback of the present decorticating machine seems to be the obtention of a large fraction of agave juice. But this juice can be recovered by mixing it with dry and minced cane stover, adding dry digestible mass to the silage or can be used for future industrial endeavors. For example, fructans present in the juice can be hydrolyzed using catalytic industrial reactors (Sakamoto *et al*.,

2020) as is currently done to produce fructose syrups in Jalisco State or they can be used to produce FOS that are being exported as high price prebiotics (Carranza *et al.,* 2015). An interesting alternative would be to use agave leaf juice as a fermentation broth for industrial production of LA. Presently, glucose from corn starch is the preferred substrate for LA production, but Mexico is a net importer of maize. Therefore, agave leaves can become an important raw material for the future production of bioplastics, such as polylactic acid, obtained by catalytic polymerization of LA (Viniegra-González, 2021).

Such considerations support the use of mechanical decortication of residual agave leaves and the consequent silage of agave pulp as a raw material for local lamb production. In this sense, agave pulp silage is a simple and useful process that can help to diversify the economics of agave plantations.

5. CONCLUSION

Here it is shown that agave pulp derived from decortication of the mature leaves of *A. salmiana* is a good substrate for lactic acid fermentation by the action of the natural microbiota present in such materials. The process does not require further optimization because this material seems to have all the nutrients required to facilitate the preferential growth of homolactic acid bacteria that transform fermentable carbohydrates present in such leaves, into a high titer of lactic acid even though there is heavy contamination by mesophilic bacteria.

It is recommended to proceed with the scale-up of the necessary decorticating equipment to provide agave pulp as the main roughage in the farmlands of Central Mexico. A subsequent outcome could be the use of residual agave juice and fibers as feedstocks for a future agroindustry based on *Agave salmiana* leaves.

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AUTHOR CONTRIBUTION

A. Ramírez-Ortiz and S.L. Alcantar-Morales performed the experiments, recorded all data, and presented the statistical analysis. D. Gallardo-Martínez, screened and supplied the LAB strains. E. Favela-Torres supervised the analytical procedures and suggested improvements to the fermentation set-ups. A.E. Cruz-Guerrero designed and supervised the experimental work and participated in the discussion and final analysis of data. G. Viniegra-González suggested the overall strategy, supervised the construction and setup of decorticating machine, developed and applied the kinetic models, and drafted the paper.

CONFLICT OF INTEREST

The authors declare no competing interests.

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