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# Maguey (*Agave spp.)* silage production with either alfalfa or mesquite pod meal as protein sources

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## 1 ABSTRACT
**Maguey (*Agave spp*) contains high sugar (242 g/kg) content and low pH (4.9), which makes it an ideal plant for silage making. However, its low protein (4.5 %) content limits its use in ruminant nutrition. The aim of the present study was to evaluate maguey silage produced with either alfalfa (A) or mesquite pods meal (MPM) as protein sources. Four different silage mixtures were produced as i) 100% maguey (M); ii) 90 % M + 10 % MPM (MM); iii) 50 % M + 50% A (MA); and iv) 33.3 % M + 33.3 % A + 33.3 % MPM (MAM). The MAM silage had the highest ($p<0.05$) dry matter content. The lowest ($p<0.05$) pH was for MAM and the highest value for MA silage. The M silage had the lowest ($p<0.05$) crude protein content. The M and MA silage, had the highest ($p<0.05$) NDF content than the other silages. The N-$NH_3$ content was higher ($p<0.05$) in MA, but had the lowest acetic acid concentration. Soluble fraction *in vitro* degradation for MAM silage was higher ($p<0.05$) than the other silages, similar results were presented for total degradation with the lowest value for M and MM silages. Combination of maguey with forages rich in protein improved silage nutritional quality and preservation was maintained.**

## 2 INTRODUCTION
Maguey (*Agave salmiana* Otto ex Salm-Dick) and mesquite (*Prosopis laevigata*), are wild natural renewable resources that are widely spread in the arid and semiarid zones in Mexico and are adapted to the adverse conditions in this environment. Mesquite pods are an important source of protein and have been used in animal and human food. The stem and leaves parts of maguey have been used for distilled beverages and sometimes the leaves are fed to ruminants (Aguirre *et al.,* 2001). However, the agave contains high levels of calcium oxalate crystals, bitter oils and saponins that limit its use as feed for ruminants (Salinas *et al.,* 2001). During the ensiling process, saponins are degraded into sapogenins, through carbohydrate fermentation that is bond to aglycon molecule. This reduces the irritation and hemolytic properties of saponins (Zamudio *et al.,* 2009). Moreover, saponins can have beneficial effects for ruminants, retaining ammonia, which improves the nitrogen utilization and stomach health (Gómez *et al.,* 2009). Fresh maguey has a high soluble carbohydrate content (242 g/kg) and low pH (4.9) (Michel *et al.,* 2008; Pinos-Rodríguez *et al.,* 2009) which favour ensiling. However, its

protein content (2.9%) is very low (Pinos-Rodríguez *et al.,* 2008a) which warrants the addition of protein sources such as alfalfa and mesquite pod meal (MPM) during the ensiling of maguey. Contrary, addition of protein sources during ensiling of maguey may impose a negative effect since they pose a high buffering capacity, causing a slow drop in silage pH. The objective of the present study was to evaluate the addition of either alfalfa or MPM on the ensilability and in vitro degradability of maguey silage.

*Agave salmiana* Otto ex Salm-Dick

Mesquite tree (*Prosopis laevigata)*

Mesquite pods

## 3 MATERIALS AND METHODS

The study was conducted in the Desert Zone Research Institute of the Autonomous University of San Luis Potosí, México. For the production of silages, leaves from mature maguey, fresh alfalfa and mesquite pods were utilized (Table 1). Maguey (M) and alfalfa (A) leaves were chopped to a 1 cm particle size by a stationary silage machine (JF40 Brasil) while mesquite pod meal (MPM) was produced by drying the material in a forced air oven at 55ºC for 24 h and milled in a Thomas–Wiley mill, using a 2 mm screen. Four different silage mixtures were produced in dry mater as i) 100% maguey (M); ii) 90 % M + 10 % MPM (MM); iii) 50 % M + 50% A (MA); and iv) 33.3 % M + 33.3 % A + 33.3 % MPM (MAM). The mixtures were ensiled by compacting with a 0.78 ton/$m^3$ density in 15 micro-silos per treatment of amber glass bottles 12.5 cm x 25 cm with hermetic caps. The silos were kept in a dark area at a room temperature of 22º C, and silos were opened after on after 0, 1, 3, 7, 10 and 60 days of ensiling. A sample of silage juice was obtained, the pH of aqueous extracts of silages was measured using a portable pH meter (HANNA model HI 8014) in 30 g silage samples blended for 30 s with 200 ml of deionized water to measure the pH. Samples were analyzed for dry matter (DM) and organic matter (OM), ash and crude protein (CP) according to the methods of AOAC (1990). Crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest *et al.,* 1991) were determined using a fibre analyzer ANKOM model A200, with filter bags ANKOM model F-57. A sample of juice from each silage was taken to determine the volatile fatty acids (VFA) concentration by acidifying with methafosforic acid in a proportion 4:1 v/v. Samples were then refrigerated ($4^0$C) for five hours and centrifuged (3000 rpm for 30 minutes). The supernatant was refrigerated and then was read in a column (0.2mm x 30m x 0.30 µm, 40 to 240/250 TR-152139) in a chromatograph gas Agilent model 6890 with a ionized flame detector at 180°C, injector of 200°C and detector 250°C; air flux 400ml and hydrogen flux of the 45 ml, and N-$NH_3$ (McCullough, 1967). The dry matter *in vitro* disappearance was performed according to Tilley and Terry (1963) technique. Ruminal fluid was used as an inoculum and was extracted from a sheep with a ruminal cannula, which was fed with a diet based on alfalfa hay and maguey silage. The rumen fluid was then mixed with McDougall saliva (1948) at a pH of 6.9; mixed on a 1:4 relation. The flasks were incubated at 39°C at 0, 3, 6, 12, 24, 48 and 72 hours. There were three runs using silage samples with three flasks for each time; with a total of 84 flasks plus another 7 flasks used as blank (flask with ruminal liquid and saliva) to correct the DM percentage of the ruminal liquid. The DM *in vitro* digestibility analysis was estimated using the model described by Ørskov and McDonald (1979):

$$P=a+b\,(1-e^{-kt})$$

Where
*P*= Degradation percentage of DM at time *t*
*a*= Soluble fraction or fast degraded
*b*= Slow degraded fraction at time *t*
*k*= Degradation constant of *b* (% $h^{-1}$)
*t*= Incubation time

The DM residual for each incubation time was adjusted to a nonlinear model using PROC NLIN (SAS, 1999). Data was analyzed as a completely randomized design. Analysis of variance and mean comparisons (P<0.05) were performed using PROC GLM and Tukey Pos-hoc-test (Steel *et al.,* 1997).

**Table 1:** Chemical composition of fresh maguey (*Agave salmiana*), alfalfa (*Medicago sativa*) and Mesquite pods (*Prosopis laevigata*).

| Item | *Agave salmiana* | Alfalfa | Mesquite pods |
|---|---|---|---|
| Dry Matter, g $kg^{-1}$ DM | 228 ± 14 | 625 ± 11 | 908 ± 19 |
| Organic Matter, g $kg^{-1}$ DM | 915 ± 22 | 886 ± 18 | 856 ± 15 |
| Crude Protein, g $kg^{-1}$ DM | 32 ± 3 | 198 ± 13 | 103 ± 9 |
| Neutral Detergent Fibre, g $kg^{-1}$ DM | 481 ± 12 | 384 ± 11 | 271 ± 13 |
| Soluble carbohydrates, g $kg^{-1}$ DM | 242 ± 24 | 58 ± 9 | 122 ± 17 |
| Ash g $kg^{-1}$ DM | 15.4 ± 3 | 145 ± 10 | 42.0 ± 7 |

## 4 RESULTS AND DISCUSSION

The results of chemical analysis of the different silages are shown in Table 2. The silages composition had effects on DM, OM, CP, NDF and Ash ($p<0.05$). The highest ($p<0.05$) DM content was found in MAM, and the lowest value for M ($p<0.05$). The DM content in the different silages varies according its composition, in M the DM is similar to the one reported by Pinos-Rodríguez *et al.,* (2009) in tender and mature maguey leaves, in the case of MM and MA, the DM concentration is similar to the one found by Pinos-Rodríguez *et al.,* (2008b) in tender and mature maguey silages, also to the one reported by Cárdenas (2003) in tropical gramineae and leguminous silages. For the case of MAM; the DM found is similar to the conventional silages forages such as maize and sorghum or other mixed silages (Cárdenas *et al.,* 2003; Danner *et al.,* 2003; Cherney *et al.,* 2004). Despite the low DM in M, MM and MA, the silages were of good quality and well preserved, since the DM requirement is 30% for a good silage fermentation and preservation (McDonald, 1981; Cañeque and Sancha, 1998). Such stability is given by high soluble carbohydrate content that maguey stores in inulin form which is rapid fermented, producing lactic acid which in turn leads to a pH reduction (Pinos-Rodríguez *et al.,* 2008a; Michel *et al.,* 2008). Silages with high DM content have high pH, and silages with high moisture content do not preserve well (Fransen and Strubi, 1998), However, the low DM content in the silages in the present study did not affect its conservation, due to low initial pH, fast fermentation of soluble carbohydrates and lactic acid production. The CP content was similar in MA and MAM ($p>0.05$), but higher than M and MM ($p<0.05$). The difference in CP depends of silage combination, in the case of M is very low, since fresh maguey has low protein content and agrees with the one reported by Zamudio et al. (2009). The highest CP silage content was for MA and MAM due to the other materials that have more protein content (mesquite pods 103 g/kg CP and alfalfa 193 g/kg CP). Protein content for MA and MAM is similar to the maize or sorghum silages according to Podkowka and Podkowka (2011). When mixing protein or nitrogen sources to the silage, the CP silage content increased at the end of the process, as reported by Díaz et al. (2001). In a study about the addition of urea to straw rice silage it was observed that adding 1.5% of urea to the silage the increased the CP content. The NDF was the same for M, MM and MA, the lowest content ($p<0.05$) was for MAM. The ADF was higher for M ($p<0.05$), than MM, MA and MAM. After 60 days pH for MM was the lowest than the other silages ($p<0.05$). The NDF content was similar for M, MM and MA ($p>0.05$) but lower for MAM which was due to the different composition of each silage. The NDF values found in this study were higher than the one reported by Pinos-Rodríguez et al. (2008a,b) and Zamudio et al. (2009) for maguey silage. In the present study the ADF content in all silages were lower than the one reported for maize or sorghum silages (McEnry *et al.,* 2006; Podkowka and Podkowka, 2011). Soluble carbohydrates in M and MM where higher ($p<0.05$) than the other treatments. The soluble carbohydrate content in M and MM was similar to the one reported by Pinos-Rodríguez et al. (2009), however, in MA and MAM it was lower. Soluble carbohydrates are the primary fermentation substrate, in some forages, an increase of these carbohydrates means that NDF and ADF are diminished (Lee *et al.,* 2001).

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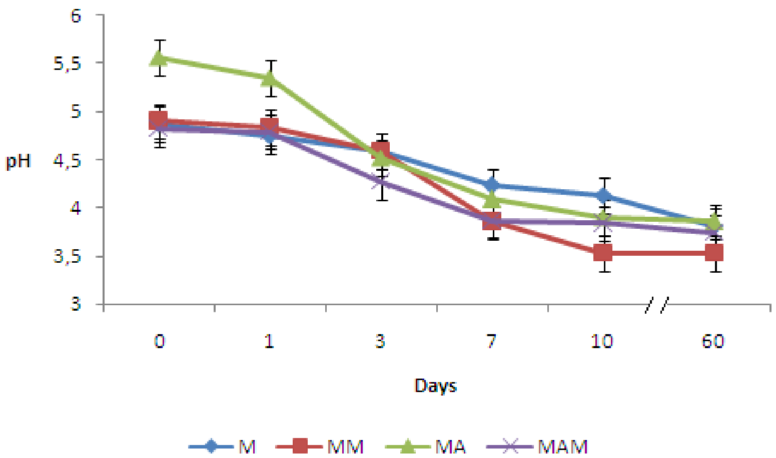
**Table 2:** Chemical composition of maguey silage produced with either mesquite pod (MM) or alfalfa (A) (n=15).

| Item | M | MM | MA | MAM | SEM |
|---|---|---|---|---|---|
| Dry Matter, g kg$^{-1}$ DM | 148.8$^{c}$ | 200.1$^{b}$ | 202$^{b}$ | 316$^{a}$ | 30.2 |
| Organic Matter, g kg$^{-1}$ DM | 846$^{c}$ | 908$^{b}$ | 856$^{c}$ | 935$^{a}$ | 61.2 |
| Crude Protein, g kg$^{-1}$ DM | 38$^{c}$ | 48$^{b}$ | 98$^{a}$ | 94$^{a}$ | 15.5 |
| Neutral Detergent Fibre, g kg$^{-1}$ DM | 553$^{a}$ | 493$^{a}$ | 535$^{a}$ | 399$^{b}$ | 34.4 |
| Acid Detergent Fibre, g kg$^{-1}$ DM | 436$^{a}$ | 379$^{b}$ | 376$^{b}$ | 272$^{b}$ | 34.2 |
| Soluble carbohydrates, g kg$^{-1}$ DM | 57$^{a}$ | 41.7$^{a}$ | 27.9$^{b}$ | 26.2$^{b}$ | 14.2 |
| Ash g kg$^{-1}$ DM | 15.4$^{a}$ | 9.2$^{b}$ | 14.6$^{b}$ | 6.5$^{c}$ | 2.8 |
| pH | 3.6$^{ab}$ | 3.5$^{b}$ | 3.7$^{a}$ | 3.8$^{a}$ | 0.1 |
| $NH_{3-}$ N g/100g TN | 2.7$^{b}$ | 7.1$^{b}$ | 14.3$^{a}$ | 5.6$^{b}$ | 2.4 |
| Lactic acid, g kg$^{-1}$ DM | 120$^{a}$ | 125$^{a}$ | 98$^{b}$ | 112$^{ab}$ | 12.9 |
| Acetic acid, g kg$^{-1}$ DM | 9.5$^{a}$ | 13.8$^{a}$ | 15.5$^{a}$ | 15.1$^{a}$ | 9.6 |
| Propionic acid, g kg$^{-1}$ DM | 2.04$^{b}$ | 2.05$^{b}$ | 2.12$^{b}$ | 7.83$^{a}$ | 0.1 |
| Butyric acid, g kg$^{-1}$ DM | 0.07$^{b}$ | 0.1$^{b}$ | 0.12$^{b}$ | 0.6$^{a}$ | 0.05 |

$^{a-c}$Mean values within the same row with different superscripts are significantly different at *P<0.05;* M: 100% maguey; MM 90 % M + 10 % MPM; MA 50 % M + 50% A; and MAM 33.3 % M + 33.3 % A + 33.3 % MPM

The silage fermentation dynamic of pH during the first ten days and at the moment of opening the glass bottles can be seen in Figure 1. The pH of MA was higher at the start and at 24 h ($p<0.05$) than in the other silages where the pH were similar ($p>0.05$). The following days of fermentation had similar pH values, at day ten M had the highest pH value and MM have the lowest value, finally at the moment to open the glass bottles there were no differences ($p>0.05$) of silages pH values. The silages pH values of the present study is in the recommended values for a good quality and well preserved silage (McDonald, 1991; Cañeque and Sancha, 1998), in all cases the pH is similar to that reported for maguey silages or maguey with alfalfa silages by Pinos-Rodríguez et al. (2008a,b) and Zamudio et al. (2009). The pH of silage is an important factor to evaluate preservation of silage. Soluble fraction *in vitro* digestibility for MAM was higher ($p<0.05$) than the other silages (Table 3) this due to a higher proportion of mesquite pod meal and alfalfa; for MM and MA no differences were found, the soluble fraction is lower for M; potential digestible fraction is similar in M, MA and MAM, in MM is lower ($p<0.05$) than M and MAM; total degradation is higher ($p<0.05$) in MAM due to alfalfa and mesquite pod meal have better digestibility, M and MM had the lowest digestibility. The highest degradation rate was for M and MM, the lowest for MAM; however, there are differences only between M and MA, MAM ($p<0.05$), even though the fibre content in these silages was lower, the degradation rate was also low.




M: 100% maguey; MM 90 % M + 10 % MPM; MA 50 % M + 50% A; and MAM 33.3 % M + 33.3 % A + 33.3 % MPM
**Figure 1:** Changes of pH throughout fermentation.

The silage content of $NH_3$-N, lactic acid, acetic acid, propionic acid and butyric acid is shown in Table 2. The $NH_3$-N as percentage of total nitrogen was high in MA indicating extensive proteolysis that was higher than in M and MAM ($p<0.05$) and similar in MM. In the case of fatty acids there were no differences in the lactate concentration in M, MM and MAM ($p>0.05$), however, it was lower for MA; similar was found for propionate and butyrate. The $NH_3$-**N** content for maguey silage is under the values reported by Pinos-Rodríguez et al. (2008b) for maguey silage; this maybe because the protein content in maguey leaves is low. In the case of MM and MAM, $NH_3$-N content is higher to the one reported in gramineae and leguminous forage silages (Cárdenas *et al.,* 2003). However, in silages where maguey was mixed with mesquite pod and alfalfa, $NH_3$-N content is higher to the one reported by Cherney et al. (2004) in different maize silages. The highest $NH_3$-N content is due to protein breakdown and $NH_3$-N concentration suggests that protein hydrolysis took place in all silages mixtures. Microbial activity contributes to the proteolysis during ensiling but this catabolic effect arises primarily from action of endogenous plant enzymes (McDonald *et al.,* 1991). It is generally recognized that the principal products of proteolysis by plant enzymes are peptides and amino acids, whereas $NH_3$-N would arise from deamination of free amino acids by silage bacteria (Oshima and McDonald, 1978; Zamudio *et al.,* 2009). Proteolysis slows down as pH medium goes down and stops when it gets to four. That is why some silage has higher soluble nitrogen content compared to a fresh green plant material (Cañete and Sancha, 1998). Lactic acid concentration in the present study is higher than the one reported in maize silage (Danner *et al.,* 2003; Cherney *et al.,* 2004), tropical gramineae and leguminous silage (Cárdenas *et al.,* 2003). The high content of maguey soluble carbohydrates allows anaerobic fermentation by the native lactobacillus that breakdown inulin and produce lactic acid (Michel *et al.,* 2008; Pinos-Rodríguez *et al.,* 2009). The acetic acid concentration was same to the values reported by Cherney and Cherney (2004); Pinos-Rodríguez et al. (2008b) in maguey silage. The presence of acetic acid in silage is important since it is an important stability indicator at the moment of open the silage (Holzer *et al.,* 2003). Soluble fraction in M is lower than the one reported by Pinos-Rodríguez et al. (2008a) in maguey silage and King grass (*Pennisetum purpureum*) silage (Anrique and Paz, 2002), total digestibility in M, MM y MA is similar to the maguey silages values reported by Pinos-Rodríguez (2008a) and in MAM is higher. VFA production in the four silages indicate that fermentation stabilization starts at day three, and from day seven through day ten does not change, which agrees with WingChing and Rojas (2007).

In the four silages, butyrate was detected, which indicate presence of pathogen bacteria i.e. *Clostridium* (McDonald *et al.,* 1991), this is for the moisture content of silages; however, all silages presented good fermentation conditions at the moment of open the silages.

**Table 3.** Means of fractions *in vitro* digestibility and degradation rate of different silages.

| Fractions | M | MM | MA | MAM |
|---|---|---|---|---|
| A | 15.1[c] | 25.1[b] | 31.4[b] | 41.7[a] |
| B | 47.5[a] | 38.7[b] | 43.0[ab] | 48.2[a] |
| a + b | 62.6[c] | 63.9[c] | 74.4[b] | 89.9[a] |
| K | 0.061[a] | 0.056[ab] | 0.035[bc] | 0.015[c] |

a=Soluble fraction, b=Potential digestible fraction, a+b= Total degradation, k=Degradation rate. [ab]Mean values within the same row with different superscripts are significantly different at $p<0.05$

## 5 CONCLUSION

Despite the low DM content, silages were well preserved. Maguey´s low pH and high carbohydrate content allows fermentation to take place more rapidly than other conventional silage. The mix of other protein sources with maguey had a positive impact on the ensiling process and improved the nutritional quality of the silage.

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