Effect of Sorghum Grain and Propionic Acid Bacteria on Fermentation Pattern, Dry Matter Loss, and Aerobic Stability of Orange Pulp Silage

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Orange pulp at 22% dry matter (DM) was ensiled in 2.7-L poly(vinyl chloride) airtight containers for 21 days to determine the effect of ground sorghum and propionic acid-producing bacteria (PAPB) on the stability of orange pulp silage. The treatments were as follows: orange pulp alone (control); orange pulp with ground sorghum grain; orange pulp with PAPB; and orange pulp plus sorghum grain and PAPB. The initial DM values of the control and sorghum grain-treated orange pulp silage were 22 and 33.5%, respectively. Total DM losses for the treatments with and without sorghum grain were 13 and 32%, respectively. After the silos were opened, the temperature in all silages increased from 22 to 31 °C over 3 days. From day 3 to day 8, the temperature of the sorghum grain-treated silage increased to about 36 °C, whereas the temperature of the treatments without sorghum grain increased to about 32 °C. Ethanol was the major fermentation product. Treatments with sorghum grain had less glucose, less ethanol, and less propionate but more lactate and crude protein than treatments without sorghum grain. Treatments with PAPB had more glucose and propionate. The addition of sorghum grain or PAPB did not improve aerobic stability as measured by changes in temperature. However, sorghum grain plus PAPB altered the fermentation pattern and may be useful additives to improve citrus pulp silage.

INTRODUCTION

Orange pulp, which is a byproduct of the orange juice industry, is readily available in large quantities in Venezuela (Wilkie *et al.*, 1990). This cannery refuse is currently a liability due to the method of disposal. The wet material is allowed to decay on nonagricultural land and is a potential environmental hazard.

Citrus pulp is highly digestible and is rich in sugars (Braverman, 1949). The in vitro digestibility of fresh pulp and citrus silage has been found to be very high, ranging between 88 and 92% (Ashbell and Donahaye, 1984), indicating the nutritive potential of the product. However, the pulp spoils very rapidly during storage and much of the nutritive content may be lost.

In the past, it was common practice to dry the peel to produce "orange peel pulp", but due to high energy costs this technology is no longer economical. The amount produced at harvesting time is more than is required for immediate use as fertilizer or animal feed. Conservation of the pulp as silage would permit its use throughout the year. However, present methods of ensiling result in high costs per unit of usable dry matter (DM) because DM losses in seepage during the ensiling process may exceed 50% (Ashbell and Donahaye, 1984). This seepage contains soluble nutrients which, if retained, could be used as a valuable feed (Braverman, 1949). Silage seepage also may create serious pollution problems, since its biological oxygen demand is very high (Mergalli and Borgoli, 1963). Seepage takes place in bunker silos when forage contains less than 30% DM, whereas in tall silos it can occur with even drier forage (35% DM) (Honig, 1980).

In conventional silage made from forage plants, such as corn or alfalfa, gas losses start with respiration by the live material and continue throughout fermentation. Because of the high amount of sugars and the low DM content of orange pulp, the fermentation dynamics are different. There is a high intensity of fermentation and gas release over the first few days. Ashbell and Donahaye (1986) collected and stored seepage separately from the mass of citrus silage. They found that seepage is not stable and its nutrient value decreases. To use orange pulp effectively for animal feed, means must be devised to reduce seepage and to arrest aerobic deterioration.

Propionic acid addition has a beneficial effect in controlling silage fermentation and has been found to reduce ammonia N formation and to limit the temperature rise in ensiled grasses, legumes, and haylage. Propionic acid also stimulates the growth of lactic acid bacteria and improves DM intake of maize silage. Studies have found that propionic acid reduces deterioration in low-DM silage and prevents deterioration in high-DM silage over a period of 19 days (Woolford and Cook, 1977). Other trials have demonstrated that higher levels of propionic acid prevent the aerobic deterioration of wet silage as compared with drier silage (Hara and Ohyama, 1978).

To ensile orange pulp economically, it is necessary to improve the fermentation process and to reduce seepage losses. Therefore, ensiling with low-moisture materials may be feasible. The objective of this study was to determine the effect of ground sorghum grain and propionic acid-producing bacteria on the fermentation pattern, DM loss, and stability of orange pulp silage.

MATERIALS AND METHODS

Experimental Procedures. Commercial fresh orange pulp (*Citrus sinensis* L.; whole pressed oranges after juice extraction) was ground in a meat grinder with a one-way $^1/_2$-in. mesh screen. The ground pulp was mixed thoroughly by hand and subjected to four treatments: pulp with no additions (control); pulp with sorghum grain; pulp with a propionic acid-producing bacteria (PAPB); and pulp with sorghum grain and PAPB. Sorghum grain was added in amounts sufficient to increase DM to 35%. PAPB was added at 10^6 colony forming units (CFU)/g of DM from a growing liquid batch culture. Treatments were ensiled for 21 days. Each treatment was replicated four times. The material was ensiled in 16 specially designed, sealed poly(vinyl chloride) plastic cylinders with approximately 2.70 kg of orange pulp in each. A one-way valve was located at the top of each container for the release of pressure. Containers were weighed once a day for the 21-day ensiling period. Weight loss was calculated from the difference between the initial weight and the weight after 21 days.

0021-8561/94/1442-0762$04.50/0

Table 1. Effect of Sorghum Grain and PAPB[a] on the Amount of Glucose, Ethanol, Lactate, Yeast, and Ammonia N and on the pH of Orange Pulp Ensiled for 21 Days

treatment	glucose (% DM)	ethanol (% DM)	lactate (% DM)	yeast (log CFU/g DM)	NH_3 N (ppm)	pH
control	0.41 (0.03)[b]	16.54 (0.86)	0.79 (0.11)	3.40 (0.25)	32.99 (2.69)	4.00 (0.17)
sorghum grain	0.09 (0.03)	7.58 (0.86)	1.16 (0.11)	2.57 (0.25)	34.30 (2.69)	3.76 (0.17)
PAPB	0.53 (0.03)	15.79 (0.86)	0.59 (0.11)	3.49 (0.25)	28.65 (2.69)	4.02 (0.17)
PAPB plus sorghum grain	0.21 (0.03)	7.77 (0.86)	1.03 (0.11)	2.81 (0.25)	44.45 (2.69)	3.73 (0.17)
control + sorghum (no PAPB)	0.25 (0.02)	12.06 (0.61)	0.97 (0.08)	2.98 (0.17)	33.64 (1.90)	3.88 (0.12)
PAPB + (PAPB + sorghum)	0.37 (0.02)	11.78 (0.61)	0.81 (0.08)	3.15 (0.17)	36.55 (1.90)	3.87 (0.12)
control + PAPB (no sorghum)	0.47 (0.02)	16.16 (0.61)	0.69 (0.08)	3.44 (0.17)	30.82 (1.90)	4.01 (0.12)
sorghum + (sorghum + PAPB)	0.15 (0.02)	7.67 (0.61)	1.09 (0.08)	2.69 (0.17)	39.37 (1.90)	3.74 (0.12)
probability						
PAPB	0.001	0.755	0.142	0.510	0.301	0.965
sorghum	0.001	0.001	0.003	0.010	0.008	0.135
PAPB × sorghum	0.999	0.598	0.714	0.753	0.019	0.873
error mean square	0.003	2.975	0.045	0.239	28.857	0.114

[a] PAPB, propionic acid-producing bacteria. [b] Least-square mean, standard error in parentheses.

On day 21, the contents of each silo were placed in plastic bags and stored open at room temperature to evaluate aerobic stability for 8 days, except for one aliquot which was stored frozen until analyzed.

Chemical Analysis. Pulp DM (fresh and ensiled) was determined by oven drying the material at 60 °C for 48 h. pH was measured in filtrates prepared from 25 g of wet material blended with 225 mL of 0.9% NaCl solution for 2 min. The filtrates were stored frozen. Samples of silage were analyzed for N by the Kjeldahl method, and crude protein was calculated by multiplying N × 6.25. Sequential detergent fiber fractions (Goering and Van Soest, 1970), ammonia (Chaney *et al.*, 1962), glucose (Feteris, 1965), and lactic acid (Barker *et al.*, 1941) were quantified. Hemicellulose was estimated as the difference between NDF and ADF. Volatile fatty acids in the above filtrates were determined using gas chromatography as previously described (Felix *et al.*, 1980). Ethanol in the filtrates was determined using high-performance liquid chromatography (Dawson, 1989).

Microbial Analysis. Serial dilutions of the filtrate were made using 0.9% NaCl. Yeast and molds were enumerated using Rose Bengal agar supplemented with streptomycin. The plates were incubated at 39 °C for 2 days, and colony forming units were counted.

Aerobic Stability. The temperature of the biomass ensiled in each silo was measured two times a day.

Statistical Analysis. Overall significance of treatments was determined by ANOVA (Gill, 1978; SAS, 1982), according to the model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{(ij)k}$$

where μ is the population mean, α_i is the PAPB effect (fixed), β_j is the addition of sorghum grain effect (fixed), $(\alpha\beta)_{ij}$ is the interaction of PAPB and sorghum grain, and $E_{(ij)k}$ is the error term (random).

RESULTS AND DISCUSSION

Residual glucose and lactic acid concentrations measured at the end of the ensiling period are given in Table 1. Glucose (percent of DM) was the lowest when sorghum grain was added, because the glucose concentration of sorghum grain is less than the glucose concentration of orange pulp. When PAPB was added, glucose was significantly higher, perhaps indicating that PAPB used glucose more efficiently. When PAPB was added along with sorghum grain, glucose was higher than that found on the treatment without PAPB. When sorghum grain was added, lactate in silage was significantly greater than control. This indicates that the addition of sorghum grain promoted a homolactic fermentation. When PAPB was added, the amount of lactate was less than the control. Perhaps the PAPB used some lactate to produce propionate.

Ethanol was the major fermentation product in the control and PAPB treatment (*ca.* 16% of the DM). A large amount of ethanol produced in the silage is typical of a heterolactic fermentation in which lactic acid and other products such as ethanol, acetic acid, and CO_2 are produced. During heterolactic fermentation of glucose, an acetyl phosphate acts as hydrogen acceptor and is reduced to ethanol (McDonald, 1981). The addition of sorghum grain reduced ethanol production, leading to a more homolactic fermentation. PAPB did not affect ethanol production.

Yeasts are associated with aerobic deterioration of silage and are known to compete with lactic acid bacteria for sugars which are fermented mainly to ethanol (Woolford *et al.*, 1979). Ashbell *et al.* (1987) concluded that yeasts apparently produce most of the ethanol and can survive despite anaerobic conditions if sufficient fermentable sugars are available to maintain their metabolism. Amounts of ethanol produced and yeast counts are in Table 1. There was less yeast present when sorghum grain was added. This may explain why the sorghum grain-treated silage had less ethanol. Citrate and malate have been detected in orange pulp in amounts up to 10 and 1.6 mg/g, respectively (Nagy *et al.*, 1977). These compounds may be a source of ethanol because citrate and malate are fermented to ethanol, lactate, and acetate (McDonald, 1981).

Ammonia N was produced in very small amounts in contrast with other experiments (McDonald, 1981). Even so, it is important to note that sorghum grain and PAPB had a significant interaction. In the treatments without sorghum grain, ammonia N concentrations were similar (Table 1). When PAPB was added to treatments with sorghum grain, ammonia N concentrations increased. The difference in the PAPB effect is explainable because the substrates are different.

The pH of all treatments after the ensiling period ranged from 3.7 to 4.0 (Table 1). There were no significant differences between the treatments. These pH values are typical of lactic fermentation and indicate that the silages were well-fermented.

Acetate was about 2% of the DM for all treatments (Table 2). The interaction between sorghum grain and PAPB was high. When bacteria were added to the treatments with and without sorghum grain, acetate was reduced. Treatments with sorghum grain and PAPB produced less acetate because more of the substrate was used for lactate and propionate production. Propionate was clearly higher in the treatments with PAPB. Perhaps the amount of propionate produced by the PAPB accounts

Table 2. Effect of Sorghum Grain and PAPB[a] on the Amount of Acetate and Propionate in Orange Pulp Ensiled for 21 Days

treatment	acetate (% DM)	propionate (% DM)
control	2.22 (0.67)[b]	0.05 (0.02)
sorghum grain	1.99 (0.67)	0.00 (0.02)
PAPB	2.17 (0.67)	0.23 (0.02)
PAPB plus sorghum grain	1.68 (0.67)	0.15 (0.02)
control + sorghum (no PAPB)	2.10 (0.05)	0.02 (0.02)
PAPB + (PAPB + sorghum)	1.92 (0.05)	0.19 (0.02)
control + PAPB (no sorghum)	2.19 (0.05)	0.14 (0.02)
sorghum + (sorghum + PAPB)	1.83 (0.05)	0.07 (0.02)
probability		
PAPB	0.020	0.001
sorghum	0.001	0.024
PAPB × sorghum	0.081	0.505
error mean square	2.018	0.002

[a] PAPB, propionic acid-producing bacteria. [b] Least-square mean, standard error in parentheses.

for the amount of lactate that was not produced when PAPB was added (Table 2). Butyrate, isobutyrate, 2-methylbutyrate, isovalerate, and valerate were not detected in the silage.

Crude protein values and sequential detergent fiber fractions are given in Table 3. The interaction between sorghum grain and PAPB was significant. In the treatments without sorghum grain, the addition of PAPB decreased the concentration of NDF. When PAPB was added to the treatments with sorghum grain, the concentration of NDF increased. The bacteria that degrade NDF could have been inhibited by propionate produced by PAPB. There is less NDF and ADF in sorghum grain than in citrus pulp. Therefore, in the treatments with sorghum grain, NDF and ADF concentrations were lower. Hemicellulose increased when PAPB was added in the presence of sorghum grain. Possibly, propionate inhibited the growth of hemicellulose-degrading bacteria. Crude protein was higher when sorghum grain was added to the citrus pulp, which is explained by the fact that the protein concentration of sorghum grain (10.8% of DM) is higher than the protein concentration of orange pulp.

Dry matter of the fresh pulp was 22%. Dry matter of the sorghum grain was 88%. Treatments with and without sorghum grain had a starting DM values of 33.5 and 22%, respectively. Dry matter concentration in the orange pulp after 21 days of ensiling ranged from 15.8 to 16.9%. Dry matter for both sorghum grain treatments after 21 days of ensiling ranged from 29.7 to 30.8% (Table 3).

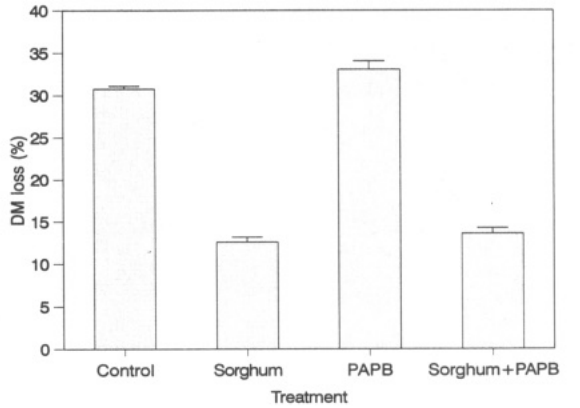

Figure 1. Effects of sorghum grain and propionic acid-producing bacteria (PAPB) on DM loss of orange pulp ensiled for 21 days.

DM losses for the treatments are given in Figure 1. These results indicate that sorghum grain led to significantly reduced DM losses in orange pulp silage under laboratory conditions throughout the ensiling period of 21 days. During homolactic fermentation, glucose is converted to lactate instead of ethanol. Therefore, less C is lost. Total DM loss for the control treatment was about one-third of the original DM of the fresh material. These losses were less than those found in other experiments (Ashbell and Donahaye, 1984), probably because the pulp in the present experiment was somewhat drier. Also, the silos used in this experiment were constructed to improve anaerobic fermentation conditions. The results are consistent with those of other experiments (Ashbell *et al.*, 1987; Ashbell and Donahaye, 1986).

A summary of weight losses of the treatments is shown in Figure 2. Sorghum grain significantly reduced the weight loss of the silage. The release of gases and seepage in the orange pulp silage were very rapid. The greatest release occurred during the initial period of fermentation. After day 5, seepage from the containers ceased. Presumably, by that time, most of the gas was released, and the weight remained stable until day 21. This result is consistent with other experiments (Ashbell and Donahaye, 1984, 1986; Ashbell *et al.*, 1987, 1989). The difference in weight loss between the treatments with and without sorghum grain was approximately 4% of the original weight. Considering that the annual production of oranges in Venezuela was 424 000 tons in 1988 (Wilkie *et al.*, 1990), the addition of sorghum could save up to 17 000 tons of orange pulp annually.

The silos were all aerobically stable over the exposure period of 8 days. Temperatures observed were lower than

Table 3. Effect of Sorghum Grain and PAPB[a] on the Amount of NDF, ADF, Hemicellulose, CP, and DM of Orange Pulp Ensiled for 21 Days

treatment	NDF (% DM)	ADF (% DM)	hemicellulose (% DM)	CP (% DM)	silage DM (%)
control	24.05 (0.27)[b]	17.74 (0.34)	6.31 (0.42)	8.93 (0.27)	16.40 (0.17)
sorghum grain	16.34 (0.27)	8.00 (0.34)	8.35 (0.42)	9.54 (0.27)	30.44 (0.17)
PAPB	23.46 (0.27)	17.23 (0.34)	6.23 (0.42)	8.82 (0.27)	16.01 (0.17)
PAPB plus sorghum grain	19.48 (0.27)	8.23 (0.34)	11.26 (0.42)	9.78 (0.27)	30.08 (0.17)
control + sorghum (no PAPB)	20.19 (0.20)	12.87 (0.24)	7.33 (0.30)	9.23 (0.19)	23.42 (0.12)
PAPB + (PAPB + sorghum)	21.47 (0.20)	12.73 (0.24)	8.74 (0.30)	9.30 (0.19)	23.05 (0.12)
control + PAPB (no sorghum)	23.76 (0.20)	17.49 (0.24)	6.27 (0.30)	8.88 (0.19)	16.20 (0.12)
sorghum + (sorghum + PAPB)	17.91 (0.20)	8.11 (0.24)	9.80 (0.30)	9.66 (0.19)	30.26 (0.12)
probability					
PAPB	0.001	0.687	0.006	0.808	0.045
sorghum	0.001	0.001	0.001	0.014	0.001
PAPB × sorghum	0.001	0.297	0.004	0.538	0.917
error mean square	0.285	0.461	0.701	0.296	0.109

[a] PAPB, propionic acid-producing bacteria. [b] Least-square mean, standard error in parentheses.

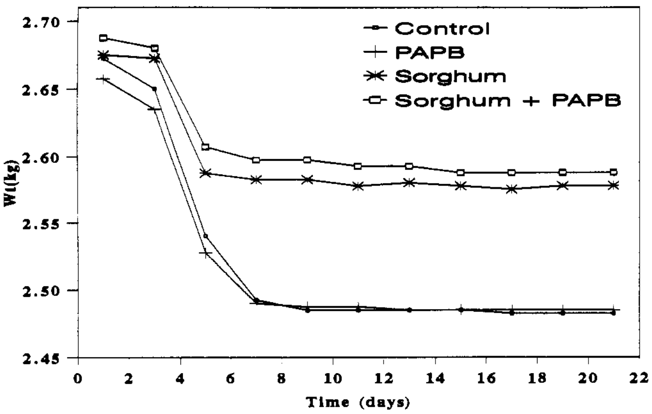

Figure 2. Effect of sorghum grain and propionic acid-producing bacteria (PAPB) on weight of orange pulp during 21 days of ensiling.

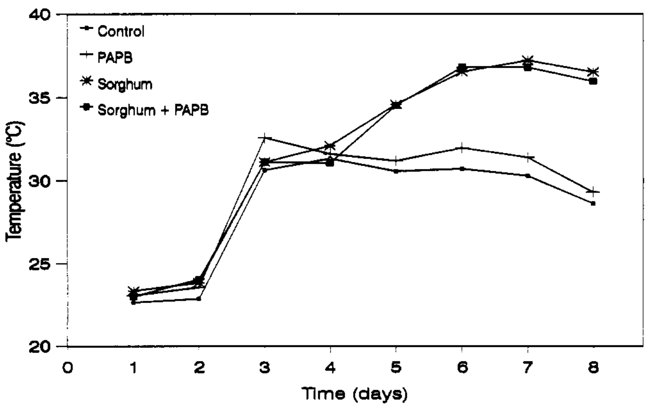

Figure 3. Effects of sorghum grain and propionic acid-producing bacteria (PAPB) on temperature changes in orange pulp silage after the silos were opened (day 1).

temperatures found in other silages (McDonald, 1981). Figure 3 shows the changes in temperatures of the ensiled orange pulp. The temperature of all treatments increased from 22 to 31 °C in 3 days. After day 3, the temperature of the treatments containing sorghum grain reached 36 °C. Aerobic oxidation of the starch may account for the temperature increase. Addition of sorghum grain or PAPB did not affect aerobic stability. Orange pulp silage had high amounts of ethanol and a low pH, which resulted in good preservation.

CONCLUSIONS

Data from this experiment demonstrate that orange pulp silage can be effectively ensiled with sorghum grain and PAPB under laboratory conditions. Field research must be performed to determine whether similar results can be achieved in commercial silos and whether such silage meets the nutritional needs of cattle.

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Received for review July 19, 1993. Revised manuscript received November 29, 1993. Accepted December 9, 1993.⊗

⊗ Abstract published in *Advance ACS Abstracts*, January 15, 1994.