Preservation of High-Moisture Maize by Various Propionate Treatments[1]

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ABSTRACT

We investigated the feasibility of using propionates produced from fermentation of maize starch to preserve high-moisture maize. The preservative effects of regular (pH 9.60), semiacidified (pH 4.86), and acidified (pH 1.70) salt solutions of a mixture of sodium propionate and sodium acetate (4.86:1.00) and of pure propionic acid were determined at propionate levels of 0.5 and 1.0% on maize harvested during 1987 at 17.6, 23.0, and 26.8% moisture. In a second test, the propionate treatments were applied to maize harvested during 1988 at 26.8 and 29.6% moisture, except the regular salt solution was replaced with either simulated fermentation broth or actual fermentation broth enriched with pure propionic acid. All propionate treatments except the regular (unadjusted pH) salt solution acted as fungicides and maintained maize in a mold-free condition for more than a year. All treatments prevented growth of *Aspergillus flavus* inoculated into the maize. Propionic acid fermentation broths were as effective as pure propionic acid. Pure propionic acid maintained the color of high-moisture maize better than did the other treatments. However, all propionate-treated samples were rated as "U.S. Sample Grade" at the end of one year of storage because of objectionable odor and high total damage.

Methods used to preserve maize include drying it to a safe moisture level (≤15.5%), storing it in oxygen-deficient or refrigerated conditions, and treating it with mold-inhibiting chemicals. However, high-moisture maize removed from oxygen-deficient or refrigerated storage molds rapidly. Fuel shortages during the 1970s and rising energy costs have stimulated interest in chemical preservation of high-moisture grain as an alternative to drying.

Several chemicals have been marketed as grain preservatives since the 1960s. Propionic acid has been shown to be an effective fungicide (Herting and Drury 1974, Sauer and Burroughs 1974, Vandegraft et al 1975) and has become accepted as the standard for judging the efficacy of other preservatives. The animal feeding value of high-moisture grain treated with propionic acid is equal to or somewhat better than that of dried grain (Ekstrom 1973, Jones 1973, Garlich et al 1976). Propionic acid has also been reported to prevent aflatoxin and ochratoxin formation from mold growth on high-moisture maize (Vandegraft et al 1975, Stewart et al 1977).

Propionic acid is manufactured by chemical synthesis from petroleum ether, but recently its production by bacterial fermentation has been considered. In this method, it is produced by various species of *Propionibacterium* that can use carbohydrates derived from maize as growth media. The predominant acids in the fermentation broth are propionic and acetic acids. Wood and Werkman (1934) achieved a total acid concentration of 21.6 g/L and a propionate-acetate ratio of 4:1 (w/w) in fermentation broth using steepwater from maize wet milling.

Industrial production of propionic acid by fermentation was advocated in the early 1920s. However, difficulties in separating the acids from the fermentation medium prevented commercial production of propionic acid by this method (Playne 1985). Although petrochemical production is at present preferred, production of propionic acid from a fermentation process could become desirable if higher petroleum costs make the synthetic route more expensive. Unpurified fermentation products may be an effective and less expensive alternative to propionic acid for grain preservation.

This study was undertaken to determine the efficacy of propionic acid fermentation products in preserving high-moisture maize. Because actual fermentation products were not available when the study was initiated, we made some assumptions about how to simulate schemes for purifying the fermentation broth. The specific objectives of the study were (1) to evaluate the ability of pure propionic acid, mixtures of sodium propionate (SP) and sodium acetate (SA) at three pH values, a simulated fermentation broth, and an enriched actual fermentation broth to preserve high-moisture maize; (2) to determine the efficacy of these chemicals on maize inoculated with *Aspergillus flavus;* and (3) to determine the effects of propionate treatments on the grade and color of maize after long-term storage.

MATERIALS AND METHODS

Maize Samples

Yellow dent maize (Pioneer 3475) was combine-harvested at 17.6, 23.0, and 26.8% moisture (wet basis) in 1987 and at 26.8 and 29.6% moisture in 1988 at the Agronomy and Agricultural Engineering Research Center near Ames, IA. The shelled maize was cleaned with a Carter-Day dockage tester with a 12/64-in. (4.76 mm) round-hole screen, and the grain was stored at 4°C for three or seven days until treatment. Moisture content was determined by AACC Method 44-15A (AACC 1983).

Propionate Treatments

The first propionate treatment was propionic acid (99% pure, pH 1.7; Kemin Chemical Co., Des Moines, IA). The second treatment was a mixture of SP and SA salts at different pH values. SP (Aldrich Chemical Co., Milwaukee, WI) and SA (Fisher Scientific Co., Itasca, IL) were dissolved in a minimum amount of water at 25°C. A weight ratio of SP to SA of 4.86:1.00 was used to make the molar ratio of the two acids 4:1, which is a molar ratio that may be expected to occur in fermentation broth. The mixture was used at (1) the regular or unadjusted pH of 9.60, (2) a semiacidified pH of 4.86 (pK_a of propionic acid), or (3) an acidified pH of 1.70 (pH of pure propionic acid). Concentrated HCl was added to adjust the pH. These treatments were selected based on the assumption that electrodialysis could accomplish significant purification of propionate and acetate salts produced by fermentation.

The third treatment employed simulated fermentation broth and was based on the assumption that if electrodialysis proved inefficient or if fouling was a problem, then another way to produce more concentrated salts would be to evaporate most of the water from the final broth. Therefore, concentrated simulated broth was used to represent the least refined case. The simulated fermentation broth was 20 times more concentrated than standard fermentation broth and contained, per liter, 50.0 g of trypticase peptone (BBL Microbiology Systems, Cockeysville, MD), 50.0 g of yeast extract (Difco Laboratories, Detroit, MI), 20.0 g of glucose, 33.0 g of NaCl, 17.0 g of K_2HPO_4, 409.6 g of propionic acid, and 81.9 g of acetic acid.

The fourth treatment was fermentation broth (350 ml) (Weier

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1989). Fermentation with *P. thoenii* strain P9 was run in batch mode for five to seven days; pH was controlled by the addition of NaOH. The initial medium contained, per liter, 6.00 g of yeast extract (Difco), 2.87 g of trypticase peptone (BBL), 30.00 ml of sodium lactate 60% syrup (Fisher Scientific), 1.67 g of NaCl, and 0.83 g of K_2HPO_4. After fermentation, the propionate concentration in the broth was 0.94%. The broth was evaporated at 63°C to 37.7 g total weight to achieve a propionate concentration of 4.84%. The ratio of acetate to propionate in the evaporated broth was 0.92:1.00. To increase the efficacy of the fermentation broth, we raised the propionate concentration to 25% while maintaining the 0.92:1.00 acetate-propionate ratio by adding 15.3 g of propionic acid and 14.1 g of acetic acid. Adding 16.8 g of water dissolved most of the evaporated and enriched broth solids. The remaining solids in the broth were dissolved by adding 127 g of a mixture of propionic acid, acetic acid, and water in the ratio of 100:92:100. Final pH of the broth was 4.1.

Propionate Application and Grain Storage

A syringe was used to add appropriate amounts of solutions to 2.1 kg of maize in a rotating mixer to reach 0.5 and 1.0% propionate (weight basis). Mixing was continued for 10 min after the propionate solution was added. Each treatment was replicated three times except for the enriched fermentation broth treatment, which was replicated two times, each time with 1.8 kg of maize.

Treated and untreated maize samples harvested in 1987 were kept in polyethylene bags (0.0018 in., 0.05 mm, thick) at approximately 23°C for 10 months. By the end of the 10th month, moisture contents of the samples had decreased several percentage points. At this time, examinations of the samples that had initial moisture contents of 17.6 and 23.0% were stopped, and distilled water was added to samples that had an initial moisture content of 26.8% to raise their moisture content to 26%. At the end of the 12th month, these reconstituted samples were transferred to a humidified chamber in double polyethylene bags (each 0.0018 in., 0.05 mm, thick). At the end of the 84th week, the samples were transferred to 1-qt (0.9-L) mason jars covered with cheesecloth to increase the availability of air to the samples.

Treated and untreated samples of maize harvested in 1988 were placed in Ziploc freezer bags, sealed, and stored in a chamber with 75–95% relative humidity at 25±2°C. Air was circulated in the chamber during storage. At the end of 32 weeks, samples were transferred to 1-qt (0.9-L) mason jars covered with cheesecloth to increase air availability to the samples.

Presence of Mold

Treated samples were tested for the presence of internal mold every 15 days during the first three months and monthly thereafter for the remainder of the storage time. Samples were collected weekly from untreated samples.

Fifty kernels from each treatment were surface-sterilized for 1 min in 1.0% sodium hypochlorite, rinsed in sterile distilled water, and then placed on malt-salt agar containing 6% NaCl (Christensen and Meronuck 1986). Plates were incubated at 25°C for seven days, and molds were then identified and counted. Molds were grown in slide culture (Taschdjian 1954) and identified according to their morphological characteristics (Raper and Fennell 1977, Samson et al 1981). Molds removed from plated moldy kernels were also examined in a drop of water under a microscope.

Inoculation of Maize

An isolate of *A. flavus* was obtained from plated moldy kernels and grown on Sabouraud dextrose agar (BBL) for six days at 25°C. Subcultures were grown in the same way to prepare spore suspensions. Surface growth of subcultured agar plates was washed off with 0.1% peptone (Difco) dilution water, and appropriate serial dilutions were made and pour-plated with acidified potato-dextrose agar (Difco) to determine spore concentration.

The maize harvested with 26.8% moisture in 1987 was inoculated four times with 1×10^2, 1×10^4, 1×10^6, and 1×10^5 spores per gram of maize (wb). The first inoculation was applied at the end of one year of storage, and the second, third, and fourth inoculations were applied on the same samples at two-month intervals thereafter.

The 1988 samples were inoculated after six months of storage. Samples with an initial moisture content of 29.6% were inoculated two times, with spore concentrations of 2.4×10^5 and 7.1×10^5 spores per gram of maize. Samples at 26.8% moisture were inoculated with 1×10^4 and 4.8×10^5 spores per gram of maize. The second inoculation was applied after samples were transferred into mason jars. For each inoculation, an untreated sample was also inoculated at the same spore concentration to serve as a control.

Color and Grade Evaluations

A Hunterlab Labscan Spectrocolorimeter (Hunter Associates Laboratory, Reston, VA) was used to determine the color of samples. Daylight was chosen as the light source, and the readings were recorded in "*Lab*" values. In *Lab* units, *L* indicates lightness, *a* indicates red (+) and green (−), and *b* indicates yellow (+) and blue (−). The greater the *a* and *b* values, the more red and the more yellow the samples, respectively. Analysis of variance for the results was done with the GLM Statistical Analysis System (SAS Institute, Cary, Inc.). Means for each propionate treatment were compared statistically by Duncan's multiple range test.

Stored samples were graded according to the Official United States Standards for corn (maize) (FGIS 1984) by the Grain Quality Laboratory, Iowa State University, Ames.

RESULTS AND DISCUSSION

Maize Microflora

The initial microflora of the maize samples harvested in the two successive years differed. Maize harvested in 1987 was initially infected primarily by *Fusarium* spp. During the first week of storage, *Penicillium* spp. began to grow. In the 1988 samples, *A. niger* was the predominant mold initially; later, *A. flavus* and *Penicillium* spp. also became abundant. *Fusarium* spp. were present but not dominant. The 1988 growing season was abnormally dry at the Ames, IA, location.

TABLE I
Mold-Free Storage Times (Weeks) for Maize Harvested in 1987 with Various Moisture Contents (MC) and Stored at 23–25°C[a]

	0.5% Application Rate			1.0% Application Rate		
Treatment	**17.6% MC**	**23.0% MC**	**26.8% MC**	**17.6% MC**	**23.0% MC**	**26.8% MC**
Control (untreated maize)	<1	<1	<1	<1	<1	<1
Regular salts	14	8	<1	36+[b]	36+	60
Semiacidified salts	36+	36+	74	36+	36+	86+
Acidified salts	36+	36+	86+	36+	36+	86+
Propionic acid	36+	36+	86+[c]	36+	36+	86+[d]

[a] Values are means of three replicates.
[b] No mold growth was observed up to observation point.
[c] One replicate of this treatment failed in the 81st week of storage.
[d] One replicate of this treatment failed in the 82nd week of storage.

Chemical Treatments

Mold-free weeks of storage for the various treatments for 1987 and 1988 samples are given in Tables I and II, respectively. Samples with mold growth on 15% or fewer of the plated kernels were accepted as mold-free. During the mold-free weeks, only samples at 17.6% moisture treated with regular salts at 0.5% reached 15% mold growth. In the rest of the treatments, mold grew on 3% or less of the plated kernels.

The regular salt solution (mixture of SP and SA at pH 9.6) was applied only on 1987 samples and was relatively slow in killing original molds. Although a 0.5% application rate was not enough to prevent the maize from spoiling, it retarded abundant mold growth for a short time at the medium and low moisture contents (Table I). At the 26.8% moisture content, the principal fungi that grew in maize treated at the 0.5% level were *Aspergillus* spp., *Fusarium* spp., and yeasts. The same molds plus *Penicillium* spp. grew at 23.0% moisture content. *Aspergillus* and *Penicillium* spp. were found at the 17.6% moisture content. The 1% application rate was sufficient to preserve maize with 26.8% moisture for 60 weeks. Failure followed the second inoculation with spores of *A. flavus*. These results agree well with those of Sauer and Burroughs (1974), who reported that maize at 22% moisture treated with 0.5% SP remained mold-free for three weeks, but maize treated with 1% SP remained mold-free for more than 17 weeks. These results indicate that the mixture of SP and SA salts at unadjusted pH (pH 9.6), even at the high application level, probably will not prevent maize from deteriorating if further contamination occurs in storage. This treatment also had the most abundant yeast growth. Because it did not show much promise, this treatment was not repeated in 1988.

The inhibitory effect of the salt solution increased with decreasing pH of the solution, as found by Bandelin (1958), because organic acids have higher antifungal activity in their undissociated forms. At pH 4.0, 88% of the propionate is undissociated, whereas at pH 6.0, only 6.7% remains undissociated (Jay 1978).

Despite four inoculations with *A. flavus,* the 1987 samples treated with 1% semiacidified salts (pH 4.86) or with 0.5% or 1% acidified salts (pH 1.70) were still mold-free after 86 weeks when the experiment was terminated. Semiacidified salts at the 0.5% application rate kept the 26.8% moisture samples mold-free for 74 weeks but failed after the fourth inoculation. The 1988 samples treated with semiacidified salts were still mold-free after 42 weeks, when the experiment was terminated. These results show that semiacidified or acidified propionate and acetate salts purified from propionic acid fermentation broth can be successfully used as grain preservatives.

Simulated fermentation broth, enriched fermentation broth (Table II), and propionic acid (Tables I and II) all showed fungicidal effects, killing natural microflora of the maize samples and providing safe storage for a long period. Failure occurred after 81 weeks of storage in only one replicate at each application level of pure propionic acid in the 1987 samples at 26.8% moisture. Among the 1988 samples, only one replicate of the 0.5% pure propionic acid treatment of maize at 26.8% moisture failed. These samples were contaminated with *A. flavus*.

No mold growth was observed in maize treated with simulated fermentation broth or enriched propionic acid fermentation broth (Table II). This result indicates that even unpurified fermentation broth can be used as a grain preservative for long-term storage.

TABLE II
Mold-Free Storage Time (Weeks) for Maize Harvested in 1988 with 26.8 and 29.6% Moisture Content (MC) and Stored at 25°C[a]

Treatment	0.5% Application Rate		1% Application Rate	
	26.8% MC	29.6% MC	26.8% MC	29.6% MC
Control (untreated maize)	<1	<1	<1	<1
Semiacidified salts	42+[b]	42+	42+	42+
Acidified salts	42+	42+	42+	42+
Propionic acid	42+[c]	42+	42+	42+
Simulated fermentation broth	42+	42+	42+	42+
Enriched fermentation broth	42+	ND[d]	42+	ND[d]

[a] Values are means of three replicates.
[b] No mold growth was observed up to observation point.
[c] One replicate of this treatment failed in the 12th week of storage.
[d] Not done.

TABLE III
Effects of 1% Proprionate Treatments on Color of Maize Harvested in 1987 and Stored for Four and Eight Months

Treatment	Storage Time (months)	Hunter Color Values[a]		
		L	*a*	*b*
Air-dried	4	61.0 a	15.5 a	28.3 a
Propionic acid	4	58.8 ab	13.5 ab	26.4 b
	8	57.3 bc	12.8 b	24.9 c
Regular salts	4	48.2 e	13.6 ab	24.7 c
	8	46.7 e	13.3 b	23.1 d
Semiacidified salts	4	55.7 cd	12.3 b	26.2 b
	8	54.0 d	12.0 b	24.9 c
Acidified salts	4	55.1 cd	11.8 b	26.4 b
	8	53.5 d	12.5 b	25.3 bc

[a] Means followed by the same letter in the same column do not differ significantly according to Duncan's multiple range test at the 5% level.

TABLE IV
Effects of Propionate Treatments on Color of Maize Harvested in 1987 and Stored for 24 Months

Treatment	Application Level (%)	Hunter Color Values[a]		
		L	*a*	*b*
Air-dried	...	48.0 a	16.6 a	23.3 a
Propionic acid	1.0	31.4 b	11.7 b	15.6 bc
	0.5	33.5 b	11.7 b	16.1 bc
Semiacidified salts	1.0	35.3 b	9.5 b	11.6 c
Acidified salts	1.0	33.3 b	11.4 b	15.6 bc
	0.5	36.9 b	11.2 b	16.9 b

[a] Means followed by the same letter in the same column do not differ significantly according to Duncan's multiple range test at the 5% level.

Color and Grade Evaluations

Tables III and IV show the Hunter colorimeter *Lab* values of 1987 samples after various storage times. The treatments bleached the samples, and the bleaching increased with increasing storage time. Air-dried maize (untreated samples of the same maize dried to 12% moisture with forced air at room temperature for about 24 hr) retained better color during storage than propionate-treated maize. Air-dried maize had the highest *L, a,* and *b* values, indicating that it was brighter, more red, and more yellow than treated maize. Maize treated with propionic acid had the highest *L, a,* and *b* values among treated samples, followed by maize treated with acidified salts and semiacidified salts during eight months of storage time. This result shows that propionic acid treatment maintained maize color better than did other treatments. Treatment with unadjusted salts gave the worst color.

L, a, and *b* values of maize kernels decreased with increasing storage time. After 24 months of storage, there were no significant differences among treatments in *L, a,* and *b* values (Table IV). Air-dried maize was significantly different from all propionate-treated samples.

The color of 1988 samples was measured after 12 months of storage. The *Lab* values did not differ significantly among treatments for maize with moisture content 26.8% (Table V). Among maize samples with 29.6% moisture (Table VI), maize treated with propionic acid had the highest *L* value, followed by maize treated with acidified salts, simulated fermentation broth, and semiacidified salts. Maize treated with semiacidified salts had slightly more red and yellow color than maize given other treatments.

The samples from each treatment stored between one and two years were graded as "U.S. Sample Grade" because of commer-

TABLE V
Effects of Propionate Treatments on Color of Maize Harvested in 1988 with 26.8% Moisture and Stored for 12 Months

Treatment	Application Level (%)	Hunter Color Values[a]		
		L	*a*	*b*
Propionic acid	1.0	36.8 a	9.4 ab	14.7 ab
	0.5	38.8 a	9.3 ab	15.9 a
Semiacidified salts	1.0	34.7 a	10.2 a	14.8 ab
	0.5	36.8 a	8.8 b	13.6 b
Acidified salts	1.0	35.5 a	10.0 a	15.3 ab
	0.5	35.6 a	9.1 ab	14.2 ab
Simulated fermentation broth	1.0	35.9 a	10.2 a	14.6 ab
	0.5	36.3 a	9.6 ab	14.7 ab
Enriched fermentation broth	1.0	35.5 a	9.6 ab	14.1 ab
	0.5	36.9 a	9.5 ab	14.0 ab

[a]Means followed by the same letter in the same column do not differ significantly according to Duncan's multiple range test at the 5% level.

TABLE VI
Effects of Propionate Treatments on Color of Maize Harvested in 1988 with 29.6% Moisture and Stored for 12 Months

Treatment	Application Level (%)	Hunter Color Values[a]		
		L	*a*	*b*
Propionic acid	1.0	31.4 a	11.1 bc	15.1 ab
	0.5	32.1 a	9.8 c	14.2 b
Semiacidified salts	1.0	25.3 bc	13.3 ab	17.8 a
	0.5	22.2 c	13.5 a	17.6 a
Acidified salts	1.0	32.5 a	10.3 c	14.0 b
	0.5	28.3 ab	11.1 bc	15.7 ab
Simulated fermentation broth	1.0	26.0 bc	13.1 ab	17.7 a
	0.5	25.6 bc	12.1 a–c	16.4 ab

[a]Means followed by the same letter in the same column do not differ significantly according to Duncan's multiple range test at the 5% level.

cially objectionable foreign odor and high total damage. The dark color of propionate-treated maize was graded as damage. Air-dried maize from the 1987 samples was graded as "U.S. No. 1."

CONCLUSIONS

The application of semiacidified or acidified solutions of SP and SA salts, simulated fermentation broth, and enriched actual fermentation broth resulted in long-term preservation of high-moisture maize. However, these treatments induced color changes in the maize samples.

Because it has already been shown that propionic acid-treated maize is appropriate for animal feed, our observations indicate that fermentation broth should be effective in preserving high-moisture maize for animal feed purposes. Use of unpurified fermentation broth may have a considerable cost advantage. Propionic-acetic acid mixtures recovered from fermentation must be at least partially acidified to be effective.

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