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# Preservation of high moisture corn by propionate treatment

Maide Ozbay Raeker
*Iowa State University*

# Preservation of high moisture corn by propionate treatment

by

Maide Ozbay

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# 1. INTRODUCTION

More than three fourths of the corn in the United States is harvested at a moisture content of greater than 20% by a combine or picker sheller because of advantages of early harvesting (Moore et al., 1973). Corn at this high moisture level is quickly attacked by spoilage organisms and therefore, must be protected.

There are preservation methods to prevent deterioration of corn such as drying of corn to a safe moisture level (lower than 13%), storing grain in oxygen limited (hermetic) or cold storage, and using mold inhibiting chemicals. The main disadvantages of hermetic storage and chilling are that the grain undergoes fermentation within a few days, molds rapidly once removed from storage, particularly after grinding or crushing for animal feed. Because of fuel shortages during the 1970s and rising energy costs, chemical preservation of high-moisture grain has gained attention as an alternative to drying.

Several chemicals have been marketed as grain preservatives since the 1960s. Propionic acid was shown as an effective fungicide (Herting and Drury, 1974; Sauer and Burroughs, 1974; Vandegraft et al., 1975) and is generally the standard for judging the efficacies of other preservatives (Sauer et al., 1975). The advantage of

propionic acid as a preservative is its low toxicity towards both humans and animals; in fact, it is a normal component of the digestive tract of ruminants (Huitson, 1968). Feeding value of high-moisture grain treated with propionic acid is equal to or somewhat better than dried grain (Young et al., 1970; Jones, 1973; Ekstrom, 1973; Herting et al., 1974; Garlich et al., 1976). Moreover, propionic acid prevents aflatoxin and ochratoxin formation in high-moisture corn (Vandegraft et al., 1975; Stewart et al., 1977).

Propionates are widely used in the food industry as inhibitors of molds and bacteria, particularly Bacillus mesentericus (Kimble, 1977). According to the U.S. Food and Drug Administration, propionates are generally recognized as safe as a direct human food ingredient and there is no limitation other than current good manufacturing practices.

Propionic acid is manufactured by chemical synthesis from petroleum ether or by bacterial fermentation. In the second method, it is produced by various species of Propionibacterium. The main products in propionic acid fermentation broth are propionic acid (PA) and acetic acid (AA) with the ratio of 2PA : 1AA. Sodium salts of these two acids are the usual products of the fermentation process.

The present project was designed to determine the effectiveness of several propionate preparations as chemical grain preservatives. Specific objectives of the study were:

1) Testing preservative properties of pure propionic acid, the mixture of sodium propionate and sodium acetate, simulation of fermentation broth, and fermentation broth on high-moisture corn; 2) Determining the effectiveness of the above chemicals in the case of any further fungal contaminations of the corn; 3) Determining the effects of propionate treatments on corn grade and color after long term storage period.

# 2. LITERATURE REVIEW

## 2.1 Microorganisms

The major microorganisms that cause spoilage in stored grains and seeds are fungi. Yeasts and bacteria can be a problem when the moisture content is in equilibrium with a relative humidity near 100%; these only play a role in the final stage of spoilage. Fungi are divided into two groups according to moisture content requirements. These groups are field fungi and storage fungi. Field fungi invade grains while they are still in the plant and after they are cut but before the grains are threshed. The major field fungi encountered on grain are species of Alternaria, Fusarium, Cladosporium, and Helminthosporium. They require a grain moisture content higher than 22% for growth. Storage fungi thrive during storage with grain moisture contents of about 13 to 20% (Christensen and Meronuck, 1986).

Development of storage fungi on stored grain is affected by moisture content of grain, temperature, storage time, amount of contamination, amount of foreign material in the grain, and activities of insects and mites (Christensen and Kaufmann, 1969).

The major storage fungi found on starchy cereal by Christensen and Sauer (1982) at relative humidities of 65-90% are shown in Table 1.

TABLE 1. Major Storage Fungi (Christensen and Sauer, 1982)

| Relative Humidity (%) | Moisture (%) | Storage Fungi |
|---|---|---|
| 65-70 | 13.0-14.0 | Aspergillus halophilicus |
| 70-75 | 14.0-15.0 | A. restrictus, A. glaucus |
| 75-80 | 14.5-16.0 | A. candidus, A. ochraceus + above |
| 80-85 | 16.0-18.0 | A. flavus, Penicillium + above |
| 85-90 | 18.0-20.0 | Penicillium, plus the above |

Bottomley et al. (1950) found that the nature of microflora varied with moisture, temperature, and oxygen concentration in stored yellow corn. As relative humidity of the air in contact with the corn was increased from 75 to 100 %, total mold count increased logarithmically. The highest mold count was found at 25 $^{o}$C. They observed that corn in equilibrium with a relative humidity of 80% supported predominantly Penicillium sp. at 25 $^{o}$C, A. flavus at 30 $^{o}$C, A. glaucus at 35 $^{o}$C and Mucor sp. at 45 $^{o}$C.

Approximate minimum, optimum, and maximum temperatures for growth of common storage fungi on grains are given in Table 2.

TABLE 2. Growth Temperatures of Storage Fungi (Qasem and Christensen, 1958)

| Fungus | Temperature for Growth, °C | | |
|---|---|---|---|
| | Minimum | Optimum | Maximum |
| Aspergillus restrictus | 5-10 | 30-35 | 40-45 |
| A. glaucus | 0-5 | 30-35 | 40-45 |
| A. candidus | 10-15 | 45-50 | 50-55 |
| A. flavus | 10-15 | 40-45 | 45-50 |
| Penicillium | -5-0 | 20-25 | 35-40 |

A rating scale was proposed by Lichtwardt and Barron (1959) for use in expressing quantitatively the degree of deterioration in samples of shelled corn collected in Iowa over a period of two years. The scale (Figure 1) was based on the percent germination of the kernels and the amount of infection by species of Aspergillus and Penicillium, which enfection they designated as the Aspergillus-Penicillium value, or simply the A-P value. The A-P value consists of the summation of the percentages of kernels infected internally by each species of Aspergillus and Penicillium in the sample. In this scale the deterioration ratings range from 0 (excellent condition) to 6 (extremely deteriorated).

A study of deterioration in stored shelled corn in Iowa during 1955 showed the presence of species of Aspergillus, Penicillium, Fungi Imperfecti, Phycomycetes, Ascomycetes,

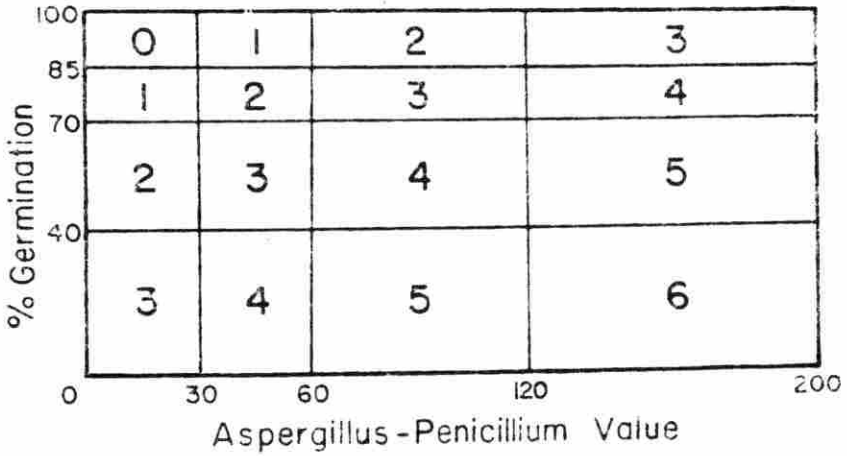



FIGURE 1. Deterioration chart for shelled corn (Lichtwardt and Barron, 1959)

Mucor, and other fungi. Aspergillus spp. predominated, however (Litchwardt et al. 1958).

Tuite (1961) determined molds from unstored corn in Indiana during 1956-58 and showed that Cephalosporium acremonium and Fusarium moniliforme were the most common species. Penicillium spp. were dominant followed by Aspergillus flavus. In a survey of corn from three midwestern mills in 1970-71, the most common fungi were Penicillium and Fusarium species. The numbers of Aspergillus, Helminthosporium, Nigrospora, and Trichoderma species were also significant (Bothast et al., 1973).

Moist corn can be stored under aeration. Although the corn cannot be preserved in this state, fungal activity is simply slowed. This is called "allowable storage time" (AST) and the deterioration of corn under this condition was modeled by complete combustion of carbohydrate:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 677.2 \text{ calories}$$

The 0.5% dry matter loss level is accepted as the criterion for the allowable storage time for shelled corn. At this level of dry matter loss, the market grade of the corn is not reduced due to kernel damage (Bern, 1983). Table 3 list days of allowable storage time for shelled corn.

The major deteriorations caused by fungi to the stored grain are decreased germination, discoloration of either the germ , embryo or entire seed, heating and mustiness, production of harmful toxins, biochemical changes within the grain, and loss in weight (Christensen and Kaufmann, 1969).

## 2.2. Corn Preservation

Grain is harvested at high moisture content to prevent harvest losses. The optimum corn moisture content at harvest for minumum loss is in the range of 28-30% (Brooker et al., 1974). This high moisture content is a favorable environment for the growth of molds and insects. Different preservation methods are available to prevent spoilage. One

TABLE 3. Allowable Storage Time for Shelled Corn for Various Grain Moisture Contents and Temperatures[a] (Bern, 1983)

| Grain Temp. °F | Corn Moisture, Percent | | | | | | |
|---|---|---|---|---|---|---|---|
| | 18 | 20 | 22 | 24 | 26 | 28 | 30 |
| | | | Days | | | | |
| 30 | 648 | 321 | 190 | 127 | 94 | 74 | 61 |
| 35 | 432 | 214 | 126 | 85 | 62 | 49 | 40 |
| 40 | 288 | 142 | 84 | 56 | 41 | 32 | 27 |
| 45 | 192 | 95 | 56 | 37 | 27 | 21 | 18 |
| 50 | 128 | 63 | 37 | 25 | 18 | 14 | 12 |
| 55 | 85 | 42 | 25 | 16 | 12 | 9 | 8 |
| 60 | 56 | 28 | 17 | 11 | 8 | 7 | 5 |
| 65 | 42 | 21 | 13 | 8 | 6 | 5 | 4 |
| 70 | 31 | 16 | 9 | 6 | 5 | 4 | 3 |
| 75 | 23 | 12 | 7 | 5 | 4 | 3 | 2 |
| 80 | 17 | 9 | 5 | 4 | 3 | 2 | 2 |

[a]The table values assume clean, combine-run corn having about 30% mechanically-damaged kernels. Mechanical damage increases the production of carbon dioxide therefore dry matter loss increases. Corn with 40% damage will have ASTs about half of the table values.

of these methods or a combination of them may be considered as means of preserving grain.

2.2.1. Drying

In this method, moisture is removed to prevent the growth of molds and insects. The required moisture content for safe storage for one year is 13% and for five years is 10-11% (Brooker et al., 1974). This method needs high

investment cost and drying-air temperatures may have a significant effect on grain quality. Excessively high kernel temperatures in corn cause increased breakage, stress cracking, and kernel discoloration, and lead to decreased starch separation (millability), oil recovery, and protein quality. The corn preserved in this way can be used for seeding, wet milling, and feeding (Brooker et al., 1974).

### 2.2.2. Sealed storage

High-moisture corn can be stored in air-tight storage units. The grain moisture content must be sufficient to provide proper respiration of the grain and associated microorganisms to consume the oxygen which exists at the beginning of storage and produce carbon dioxide to prevent deterioration of the grain.

Corn removed from sealed storage must be used within 24 to 48 h to avoid deterioration. Grain from sealed storage is used for feeding and is usually stored in the same area as produced and consumed (Brooker et al., 1974).

### 2.2.3. Cooling or chilling

The respiration of the grain and of the microorganisms is reduced as the temperature of the storage decreases below 4 $^{o}$C. Thus, by reducing the temperature of wet grain the storage life is increased (Brooker et al., 1974).

2.2.4. Chemical treatment

The environment surrounding the microorganisms can be made unfavorable for their growth by applying certain chemicals.

Organic acids such as propionic, acetic, formic, sorbic, butyric, isobutyric and their salts have been used as grain preservatives since the late 1960s. Grain with moistures in the range of 20% to 30% can be maintained in good condition up to eight months under proper conditions (Deyoe et al., 1973). A chemical should have the following properties to be accepted as a grain preservative: be effective over a wide range of moistures and temperatures; be cost effective at application rates; be reasonably safe to handle and require minumum investment for both application and storage; produce no toxicity or palatability problems as feed (Sauer and Burroughs, 1974). Table 4 gives a summary of the effectiveness of the chemicals with some other commonly used compounds.

Organic acid is applied to grain immediately after harvest by a spray nozzle in a closed chamber. Subsequent mixing in an auger tube applies acid to every kernel. The pH of the kernel is lowered to about 4.5, and fungi and seed germination are killed (Hall et al., 1974).

TABLE 4. Some Common Feed and Food Preservatives (Wilcox, 1985)

| | |
|---|---|
| Propionic acid | A very effective grain preservative, Widely tested. Used in food preserving. Pungent odor. Calcium and sodium propionates appear less effective than the acid form. |
| Acetic Acid | About half as effective as propionic acid at 16 to 20% moisture levels and less effective at high moisture levels (over 25%). Appears to be more effective as a preservative when combined with propionic acid. Much used in foods as for pickling and "curing". Pungent odor- the "vinegar smell" of vinegar. Calcium and sodium salts have no preserving effect. |
| Isobutyric Acid | Tests indicate effective preserving effect on grain. Quite pungent odor. |
| Ammonium Isobutyrate | Tests indicate effective preservative effects. Less pungent than acid. |
| Formic Acid | Has preserving effect which appears about equal to acetic acid. The liquid or vapors are dangerously caustic to skin and body tissues. Very pungent odor. Calcium formate is effectively used in silages in Europe. |
| Lactic Acid | Some preserving effect in high-moisture foods. Needs further testing as grain preservative. |
| Sorbic Acid | Along with potassium sorbate is used in bakery doughs to inhibit molds and yeasts. Effective grain preservative when dissolved in alcohol and throughly distributed on the grain. Very insoluble in water solutions. |
| Benzoic Acid and Sodium Benzoate | Preservative for foods and fats. Limited by law to not more than 0.1% in food or feed. Needs further testing for grain preservation. |

Reasons involved in the deterioration of organic acid treated high-moisture grain stored for long period are: nonuniform treatment causes pockets of untreated or partially treated corn where "hot spots" occur and spread throught the bin; changes in ambient temperature result in condensation of water vapor; sufficient oxygen and appropriate temperature allow fungi growth; application doses lower than effective levels do not inhibit mold growth (Deyoe et al., 1973); and the nature of the microflora invading the grain affects the preservation time of the treatment. Field fungi and Gram-negative bacteria are the most sensitive to the treatment. Among molds, Penicillium especially grows on grain when the propionic acid level is below the effective concentration (Poisson and Cahagnier, 1972).

The treatment level may be lowered to below the concentration required to prevent fungal growth by the movement of condensed water vapor and water released by respiring fungi. Moving condensed water can transport mold spores produced by pockets of untreated corn (Smith and Stevenson, 1975). In addition, some degradation of organic acid may result from microbial activity as suggested by Burrell et al. (1973). These effects cause rapid spread of fungi in the presence of sufficient oxygen and temperature.

2.2.4.1. Inhibition mechanisms of organic acids

It has been known for some time that organic acids possess antimicrobial activity and the mechanism of action of organic acids has been studied by several investigators. Samson et al. (1955) demonstrated that acetate and short-chain fatty acids inhibited phosphate uptake and accumulation, as well as, respiration of S. cerevisiae at pH of 4.5. They stated that the inhibition effect of acetate was due to interaction of the fatty acids or anions with certain enzymes and proteins of the yeast. Fencl and Leopold (1957) investigated respiratory inhibition in A. niger with acetic acid. The authors stated that spore germination was inhibited by undissociated acetic acid and the inhibition site was at the cell surface. According to their explanation, the potential of the cell membrane was changed because of fixation of acetic acid to the cell surface. Therefore, the membrane became impermeable to anions or enzymatic systems taking part in the transport of anions from the medium into the cell were inhibited.

Strider and Winstead (1960) indicated that the site may differ with different fungi. Their results with C. cucumerinum showed that the site of action was within the cell, whereas with A. flavus it was at the cell surface.

2.2.4.2. Application rates and effectiveness of chemicals

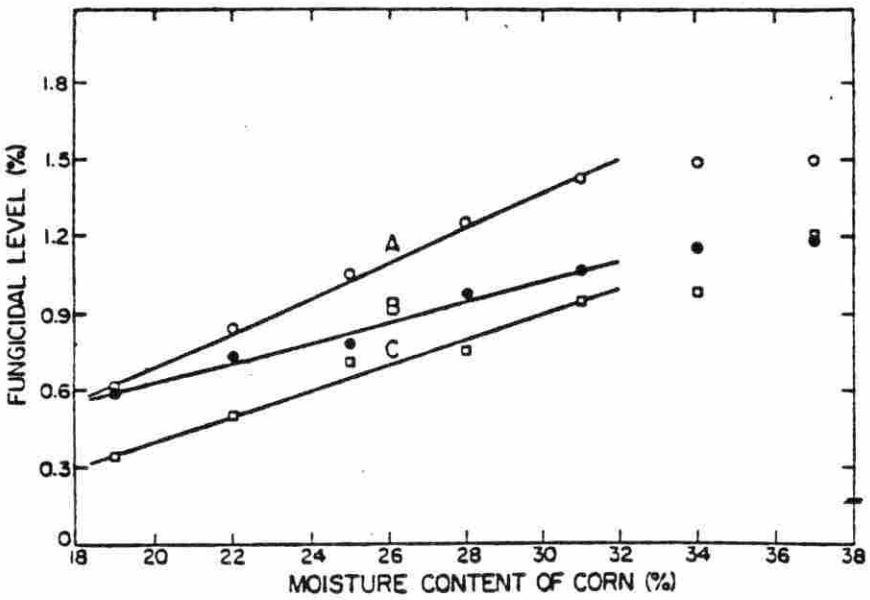
The chemical concentration required depends on the moisture content of the grain to be protected, the storage conditions, and the desired length of storage. Experimental results show some conflict between laboratory conditions and large-scale conditions with respect to effective fungicidal level of chemicals.

Effective fungicidal level (EFL) of organic acids and their binary and ternary mixtures was determined at several moistures by Herting and Drury (1974). It was found that mixtures of acids were usually more effective than any single one. Table 5 shows the effective fungicidal level of acids and mixtures. The EFL level is the required minimum level to inhibit microorganisms. Temperature and relative humidity were 30 $^{o}$C and 70-90% respectively in the experiment. Since positive air pressure was used to provide enough oxygen for fungal growth, the authors indicated that aeration might increased EFL level because of volatilization of tested compounds. The EFLs were sufficient to maintain the samples mold-free for a year and also prevented the growth of A. flavus inoculated into the grain. Organic acids prevent formation of toxins by inhibiting toxin-producing fungi. The EFL increased with increasing moisture contents. This relationship is shown in Figure 2.

TABLE 5. Minumum Fungicidal Level of Acids and Mixtures on Corn at 20% Moisture Content (Hertung and Drury, 1974)

| acid | EFL (g/100g) |
|---|---|
| Formic | 0.64 |
| Acetic | 1.06 |
| Propionic | 0.80 |
| Butyric | 0.71 |
| Isobutyric | 0.56 |
| Acetic:propionic (1:1) | 0.88 |
| Acetic:propionic (3:2) | 0.80 |
| Acetic:butyric (1:1) | 0.61 |
| Acetic:isobutyric (1:1) | 0.78 |
| Propionic:butyric (1:1) | 0.43 |
| Propionic:isobutyric (1:1) | 0.33 |
| Butyric:isobutyric (1:1) | 0.41 |
| Formic:acetic:Propionic (3:3:4) | 0.47 |
| Acetic:propionic:butyric (1:1:1) | 0.52 |
| Acetic:propionic:isobutyric (1:1:1) | 0.52 |
| Acetic:butyric:isobutyric (1:1:1) | 0.60 |
| Propionic:butyric:isobutyric (1:1:1) | 0.34 |

Water addition to volatile fatty acids increased their antifungal activity. Therefore, the required levels for aqueous dilutions of acids were lower than those of individual or mixed acids (Hertung et al., 1974). The explanation for this finding was the conversion of acid-acid dimers to acid-water dimers. Table 6 shows the effect of dilution on amount of acid required for a fungicidal effect at 20% moisture corn. As shown in Table 6, the required amount of acid is approximately constant with increased of water in the dilution until the water content of the




Regression equations: A, propionic acid, EFL = −0.65 + 0.067(% moisture); B, isobutyric:propionic acids (90:10), EFL = −0.18 + 0.040(% moisture); C, isobutyric:propionic acids (60:40), EFL = −0.58 + 0.049(% moisture).

FIGURE 2. Effect of moisture content of corn on the effective level (Hertung and Drury, 1974)

mixtures exceeds 40%. Then the required amount of acid decreased.

The recommended application rates of organic acids found by Hall et al. (1974) at different moisture contents for long term storage are listed in Table 7.

Deyoe et al. (1973), Sauer and Burroughs (1974), Goering and Gordon (1973), and Paster (1979) compared the preservative effectiveness of various organic acids and their salts under laboratory conditions. Among the tested compounds, propionic acid exhibited the highest fungicidal

TABLE 6. Effect of Dilution on Amount of Organic Acids required for Fungicidal Activity in Corn Having 20% Moisture (Hertung et al., 1974)

| Composition of Formulation | | | |
|---|---|---|---|
| Water (%) | Organic Acid[a] (%) | Effective Fungicidal Level (g/100g) | Organic Acid Required (g/100g) |
| 0 | 100 | 0.34 | 0.34 |
| 10 | 90 | 0.34 | 0.31 |
| 20 | 80 | 0.43 | 0.34 |
| 30 | 70 | 0.52 | 0.36 |
| 40 | 60 | 0.61 | 0.37 |
| 50 | 50 | 0.52 | 0.26 |
| 60 | 40 | 0.70 | 0.28 |
| 70 | 30 | 0.61 | 0.18 |
| 80 | 20 | 0.88 | 0.18 |
| 90 | 10 | 1.67 | 0.17 |

[a]Ternary mixture of propionic:butyric:isobutyric acids (50:25:25).

activity and at the treatment level of 0.4% was effective more than 35 weeks on corn having 22% moisture stored at 23°C. Sorbic acid was an effective mold inhibitor for high-moisture grain when dissolved in ethanol. Salts of acids tended to be less effective. Some results of these experiments at 27°C are summarized in Table 8.

TABLE 7. Recommended Acid Application Rates for sheeled Corn, Percent Acid by Weight (Hall et al., 1974)

| | Propionic (%) | Acetic (%) | Storage Time | Corn Moisture Content (%) 20 | 25 | 30 | 35 |
|---|---|---|---|---|---|---|---|
| Cleanese[a] "Chem Stor" | 80 | 20 | 1 yr | 0.90 | 1.20 | 1.50 | 1.75 |
| Chevron "Ortho Preser." | 100 | 0 | 3 Months | 0.34 | 0.45 | 0.70 | |
| | | | 6 Months | 0.45 | 0.70 | 0.89 | |
| | | | 8 Months | 0.70 | 0.89 | 1.00 | |
| British Petroleum "Propcorn" | 100 | 0 | 1 yr | 0.75 | 1.00 | 1.25 | 1.50 |
| Union Carbide "Sentry No. 1" | 100 | 0 | 1 yr | 0.75 | 1.00 | 1.25 | 1.50 |

[a]Formerly 40% propionic and 60% acetic acid.

TABLE 8. Mold-Free Storage Time (Weeks) for Corn Having Moisture Contents of 18%, 20%, and 22% and Stored at Approximately 27° (Sauer and Burroughs, 1974)

| Chemical Applied | 0.5% Application Rate | | | 1% Application Rate | | |
|---|---|---|---|---|---|---|
| | 18% | 20% | 22% | 18% | 20% | 22% |
| None | Less Than One Week | | | | | |
| Potassium Sorbate | 2 | 1 | 1 | 3 | 2 | 1 |
| Sorbic Acid | 7 | 2 | 1 | 12 | 5 | 1 |
| Calcium Propionate | 1 | 4 | 0 | 1 | 10 | 10 |
| Sodium Propionate | 17+ | 5 | 3 | 17+ | 17+ | 17+ |
| Acetic Acid (Glacial) | 17+ | 17+ | 7 | 17+ | 17+ | 12 |
| Propionic Acid | 17+ | 17+ | 17+ | 17+ | 17+ | 17+ |

The required levels for propionic acid to give succesful preservation in grain sorghum were 0.75% at 26% moisture content (Nelson et al., 1973) and 0.5% at 16% moisture content (Paster, 1978).

An experiment was conducted by Bothast et al. (1975) to test the preservative effects of ammonia, ammonium isobutyrate, isobutyric acid, and propionic-acetic acid on large scale, 52.8 $m^3$ lots of freshly harvested corn with a moisture content of 27%. Application rates were 0.5%, 1.75%, 1.5%, and 1.2%. Since organic acids had a fungicidal property and acted almost as bacteriostats, they were superior to the other two chemicals. However, A. flavus growth was observed in acid treated samples during a long

storage time. Burrell et al. (1973) concluded that although 0.4 and 0.5% treatment levels were sufficient for propionic acid to prevent mold growth under laboratory conditions, they would not be effective in large scale because of condensation in the bin. They stated that these application rates could be used as backup for drying in bin.

Extrom (1973) tested organic acids for their preservative effectiveness on corn having a moisture level in the range of 25-35% for long-term storage in Sweden. The recommended doses of propionic acid and formic acid by weight were between 0.80 and 1.20%, and between 1.30 and 1.80%, respectively. Suggested application concentrations of propionic acid in Norwegian conditions closly resemble those of Sweden. Recommended concentrations were between 1.0 and 1.2% for long-term safe storage and a 0.3% applicaton level was sufficient for only three months of storage (Sogn, 1973). In contrast with previous studies, effective doses were higher in Indiana. Singh et al. (1987) used propionic acid and a mixture of acetic:propionic:butyric (40:40:20) on wheat at a moisture content of 18%. They found that 1.5% application rate was not effective, but 5% suppressed fungal growth for six months, at which time A. flavus contamination occurred.

Bernier (1973) treated wheat, triticale, barley and oats with formic acid, acetic acid, propionic acid, and a mixture of acetic and propionic acids in 1:1 and 7:3 (v/v) ratios and stored the grain at 22 to 28 °C for 12 to 14 months. Three moisture contents (20%, 25%, and 30%) for each grain were used. Results showed that all acids were effective at levels of 0.5% on oats, wheat, triticale and barley at a moisture content of 25%. In general, it was stated that propionic acid and acetic:propionic (1:1) were most effective.

Methylene bis propionate (MBP) was examined for preservative efficiency on high-moisture corn (Sauer et al., 1975; Bothast et al., 1978). MBP exhibited superior antifungal and antibacterial effects to propionic acid during storage. Although low mammalian toxicity, less corrosiveness, and superior preservative efficiency were advantages of MBP according to feeding trials, it decreased average daily gain. The reason for that was the breakdown of MBP into propionic acid and formaldehyde within 9 h after treatment. Formaldehyde in the MBP ration was implicated to cause decreased performance.

Smith et al. (1983) carried out an experiment to determine the effects of temperature on propionic acid preservation. Three treatment levels (0.025, 0.05, and

0.1%) and two storage temperatures (room temperature and 5°C) were used. A level of 0.1% propionic acid was effective in inhibiting mold growth at 5°C but was not effective at room temperature.

Muller and Thaler (1981) inoculated propionic acid treated corn with spores of 7 Fusarium species, 6 yeast species, Paecilomyces varioti and Trichoderma viride. Moisture contents of samples were adjusted to 19, 25, 32, and 40%, and application dosages were 0.3, 0.5, 0.7 and 1.0% respectively. All treatment levels inhibited inoculated fungi growth and toxin production by strains of Fusarium for 6 months at 20°C. They concluded that sufficient doses of propionic acid to inhibit fungal growth were lower than those recommended in the literature for large-scale treatment of corn. Sherwood and Peberdy (1974) came to the same conclusion. They examined the effects of propionic and other organic acids on the growth of Fusarium and the production of the estrogenic mycotoxin zearalenone. Their results showed that a 0.1% application level was not enough to inhibit fungal growth but 1.0% treatment level was. They stated that in practice, recommended propionic acid doses were approximately five times higher than the concentration sufficient to prevent Fusarium growth. This was due to the technical difficulties of uniform distribution of the acid on all kernels.

Stewart et al. (1977) conducted an experiment in which they tested various antifungal agents in a medium consisting of 2% yeast extract and 4% sucrose. They inoculated A. parasiticus into the medium and incubated at 26°C for 10 days. Propionic acid and crystal violet were the most effective compounds in inhibiting fungal growth. It was found that propionic acid was fungicidal at concentrations greater than 3.0 g/ml. Although calcium propionate did not inhibit fungal growth, it slightly decreased aflatoxin production. Sodium propionate was ineffective in both ways.

Storage studies done with urea, potassium sorbate, and potassium sorbate plus propylene glycol show that these chemicals had some mold-retarding properties. There was, however, significant discoloration of the grain and in the latter two, an increase in kernel breakage susceptibility (Ghate and Bilanski, 1981; Yasin and Hanna, 1989).

2.2.4.3. Biochemical changes in corn during different ways of conservation

Chemical changes occur in grain with varying rates which depend upon storage conditions. The action of grain enzymes and fungal growth result in the changes. Increased temperature and moisture increase enzymatic and respiratory activity so that these changes occur rapidly and cause deterioration (Geddes, 1958).

In a project undertaken by Bottomley et al. (1952), the effects of aeration, time, and moisture content on fat acidity, nonreducing sugars and mold flora of stored yellow corn were investigated. During storage, the mold count and fat acidity increased, nonreducing sugar and seed viability decreased as moisture increased. Decreases in nonreducing sugars were more closely related with mold count than were increases in fat acidity. While the growth of A. glaucus rapidly decreased nonreducing sugars, the growth of A. flavus, A.candidus, Penicillium sp., and Fusarium sp. increased fat acidity. Changes were slight in non-aerated samples, but seed viability decreased.

The loss of carotene in maize harvested at 22.5% moisture content and treated with acetic and propionic acids was investigated by Simon et al. (1972). Carotene content in treated and untreated samples sharply decreased within 24 h in maize. Although the initial drop was greater in treated samples, later on this drop was slower in treated corn. Propionic acid treated samples at the level of 0.6-0.8% lost 36% of carotene in 1 week, and 45% in 3 months. Treatment with acetic acid at a level of 0.9% resulted in a 55% loss of carotene in 3 months. They concluded that increases in acid application doses up to the required level result in more effective mold control, a

slower decrease in carotene content and lower fatty acid value. A greater than required level increased carotene loss.

Alexeeva et al. (1982) examined biochemical changes in maize stored at temperatures of 10°C, 15 to 20°C, and 25 to 30°C. Yellow dent maize from the 1979 crop having moisture contents of 20.6%, 24.2%, and 28.2% was stored for 2 months at the above temperatures in different ways. Maize was: treated with propionic acid at the level of 1% /PA/, or; stored in controlled atmosphere (97.0-97.5% nitrogen, 1.5-2.0% carbon dioxide, and 1% or less oxygen) /CA/; stored untreated as a control. PA treatment preserved the original color of maize at all temperatures. CA was effective on color retention for moisture contents of 20.6 to 24.2% at temperatures not exceeding 20°C. The samples stored in CA with the exception of the lowest moisture and temperature had a distinct smell of ethyl alcohol in the grain. Dry matter losses were highest in control grain followed by CA storage. Respiration level was very low in PA-treated samples and the production of carbon dioxide was 2.64 to 9.07 mg/100g of dry matter during 24 h. While the amount of reducing sugars remained stable in PA treated samples, they decreased in the control and CA stored samples. This was attributed to respiration of the grain. There was an

increase in free fatty acids and diglycerides with a simultaneous drop in triglycerides in control samples. Diglyceride content did not change in PA treated samples but there was some increase in CA-stored samples. PA treatment also prevented lipid oxidation while others did not.

2.2.4.4. Feeding value of acid treated grain

Acid-treated grain can be used only as animal feed because Food and Drug Administration regulations have not approved it for other uses (Bern, 1988). Numerous feeding studies have shown that the sour taste and pungent odor of acid do not have negative effects on the feed intake by pigs, cattle and laying hens.

Bayley and Holmes (1972) reported that diets containing acid-treated corn increased retention of energy and nitrogen in comparison with dried corn. A possible explanation for these observations was offered by Jones et al. (1974). Their results showed that acid preservation of high-moisture corn caused some hydrolysis in starch molecules during storage. Therefore, treated corn was partially "pre-digested" before feeding. It was also found that retention time of acid-treated corn in the stomach was greater than that for dry corn. These two factors increased digestibility of high-moisture corn and, therefore, nutrient utilization was improved. These factors result in equal or

better feed value of acid-treated high-moisture corn compared to dry corn. Bern (1988) compared feed values of acid-treated corn versus dry corn (Table 9).

TABLE 9. Feed Value Comparison (Bern, 1988)

| Animals | Parameter | Relative Values Dry Corn | Acid Corn |
|---|---|---|---|
| Finishing beef | Daily Gain | 100 | 102.7 |
| | wt gain/wt feed | 100 | 103.7 |
| 75-lb Feeder pigs | Daily Gain | 100 | 106.6 |
| | wt gain/wt feed | 100 | 100 |

2.2.4.5. Cost comparisons of various grain preservation methods

The costs for different corn storage systems have been evaluated. These estimates show that acid treatment is quite competitive with alternative methods such as drying and ensiling. The major advantage in chemical preservation is low investment cost. Because of a multiplicity of individual variations, such as the prices for equipment and facilities, the equipment's rate of depreciation, and the labor requirements, they may not represent actual costs for any particular farmer.

An approximate cost comparison for different systems was made in Illinois for the fall of 1973 by Hall et al. (1974). Table 10 summarizes this cost comparison.

TABLE 10. Costs per Bushel for Handling, Conditioning, and Storing Corn[a] (Hall et al., 1974)

| Annual Volume, (bu) | System | | | | | |
|---|---|---|---|---|---|---|
| | Bin Dryer | Batch Dryer | Acid in Bin[b] | Acid Under Plastic[b] | Acid, no Storage[c] | Acid Elevator |
| | Cents per Bushel | | | | | |
| 5, 000 | 24.92 | 36.83 | 27.10 | 24.30 | 16.95 | 23.00 |
| 10, 000 | 18.33 | 21.93 | 23.10 | 18.40 | 15.25 | 23.00 |
| 20, 000 | 15.93 | 20.03 | 21.70 | 15.90 | 14.05 | 23.30 |
| 50, 000 | 13.83 | 16.23 | 19.70 | 14.10 | 12.35 | 23.30 |
| 100, 000 | 13.23 | 15.63 | 19.00 | 13.40 | 11.75 | 23.30 |

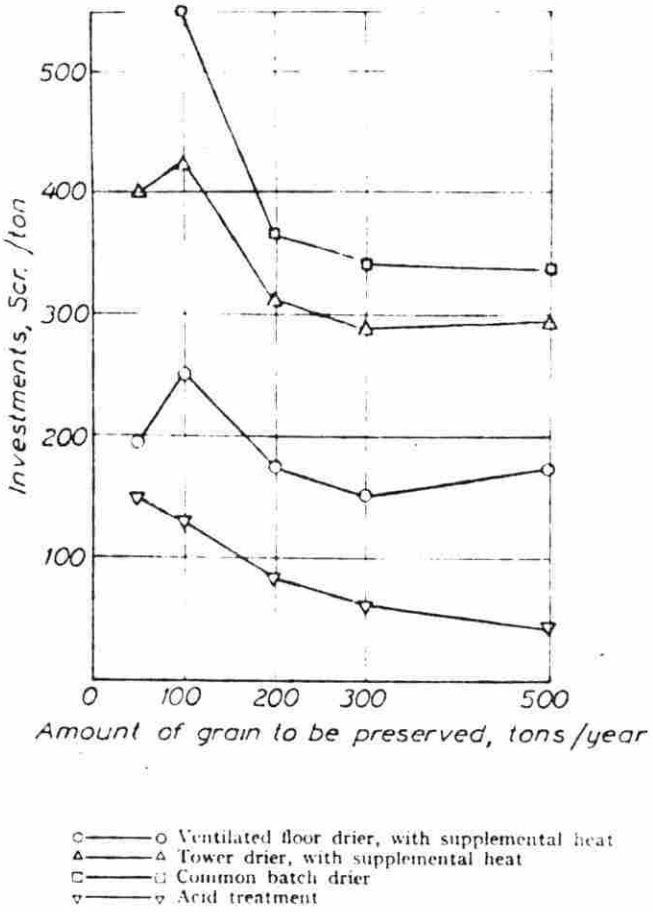
[a]Includes fixed and variable costs of facilities and operation, losses in the field, and shrink. All systems assume the purchase of new equipment and facilities. Fuel costs are based on LP gas at 30 cents per gal. Acid is priced at 26 cents per lb and applied at the rate of 1.1% by weight.

[b]Costs of handling high moisture corn are reduced by 8 cents per bu, as an assumed increase in feed efficiency of 4%.

[c]Costs of handling high moisture corn are reduced by 8 cents per bu, as an assumed increase in feed efficiency of 4%. Acid treatment without storage facilities is recommended only in low rainfall climates or during periods of relatively low rainfall and temperature.

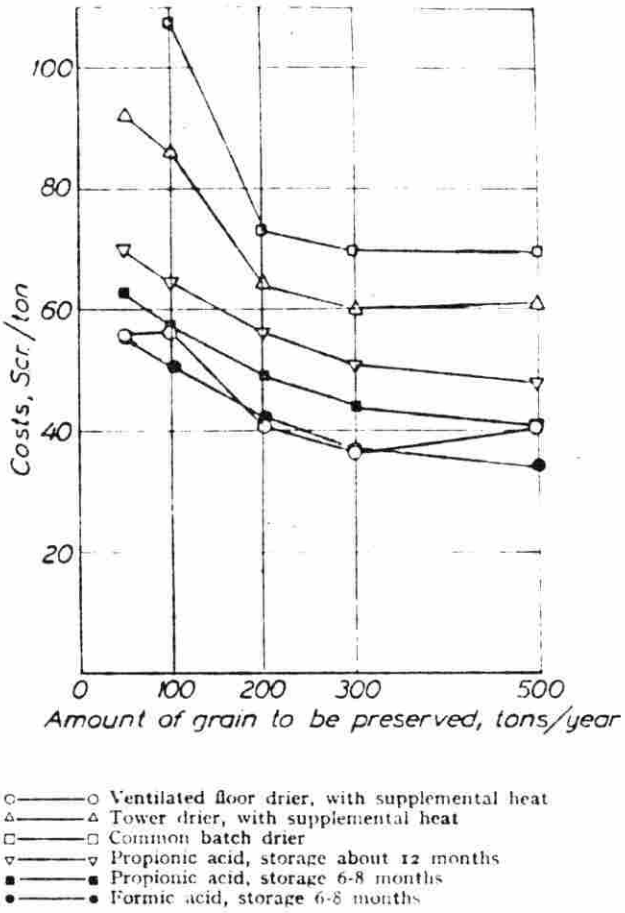
Another cost evaluation study was conducted in Sweden by Extrom (1973). He compared various drying systems with acid treatment on grain having 25% moisture. Since acid-treated grain could be stored in existing buildings, investment cost was very low for acid treatment as shown in Figure 3.

The price of the preservative is an important factor in cost comparison. The cost of the acid caused the total cost of the treatment to be approximately as large as the cost of drying in simple grain driers (Figure 4).




Conversion of Swedish units (scr) to dollar ($):
100 scr = 15 $ 200 scr = 30 $
300 scr = 45 $ 400 scr = 60 $
500 scr = 75 $.

FIGURE 3. Calculated Investment Costs for Various Storage Systems (Extrom, 1973)




Conversion of Swedish units (scr) to dollar ($):
20 scr = 3 $ 40 scr = 6 $
60 scr = 9 $ 80 scr = 12 $
100 scr = 15 $.

FIGURE 4. The Calculated Costs for Drying and Acid Treatment of grain at moisture content of 25% (Extrom, 1973)

# 3. MATERIALS AND METHODS

## 3.1. Corn

This experiment was run two successive years, 1987 and 1988. In September and October, yellow dent corn (Pioneer 3475) was harvested at different moisture contents by combine at the Agronomy-Agricultural Engineering Research Center located near of Ames, Iowa. The moistures at harvest were 17.6%, 23.0%, and 26.8% in 1987 and 26.8% and 29.6% in 1988. The shelled corn was cleaned with a carter dockage tester having a 12/64-in. round hole screen to remove broken kernels and foreign materials. Cleaned corn was stored at $4^{o}$C for 3 or 7 days until treatment.

The steps of this experiment were:

Preparation of chemical solutions;

Determination of moisture content of newly harvested corn;

Application of chemicals to corn;

Determination of moisture content of treated corn;

Storage of treated and untreated corn;

Collection of samples for analysis;

Examination of mold growth in corn by plating of corn on media;

Inoculation of the corn; and

Determination of the corn color after storage

## 3.2. Treatments

The goal of this experiment was to test the fermentation broth produced by propionic acid fermentation as grain preservative. The basic products from the fermentation are propionic acid and acetic acid with the ratio of 2 moles to 1 moles, respectively. Since actual fermentation products were not available when the experiment started, six different treatments were used to mimic different purification schemes of the fermentation broth.

One potential way to purify the broth is to use electrodialysis. In this case, we expected significant purification of the propionate and acetate salts that are produced. The larger peptides and protein molecules and the uncharged sugar molecules should not pass through the electrodialysis membrane. Therefore, this situation could be simulated quite well with the mixture of sodium propionate and sodium acetate salts. The weight ratio of sodium propionate to sodium acetate was 4.86:1.00 in order to make the molar ratio of the two acids 4:1. The salts were applied at the following pH values by adjusting with HCl:

Salt solution of propionate and acetate (pH 9.60);

Semi acidified salt solution (pH 4.86, the pKa of propionic acid); and

Acidified salt solution (pH 1.70, the pH of pure propionic acid)

Another assumption was that electrodialysis would not be done. If electrodialysis proves to be too inefficient, or if fouling is a problem, another way to produce more concentrated salts would be to just evaporate most of the water from the final broth. As long as it is at neutral pH there should be little problem with volatile losses of propionic or acetic acids. This scenario would also result in the "dirtiest" sample, because all nonvolatile broth constituents would be concentrated as well. Therefore, concentrated simulated broth was used in this experiment to represent the "dirtiest" case.

Fermentation broth from propionic acid fermentation was also tested. Since the amount of broth and its propionate content were insufficient for treatment, some propionic and acetic acid additions were necessary.

Pure propionic acid was used as a control treatment. Thus, the comparison of the above treatments with propionic acid was be possible.

Since the salt solution treatment (pH 9.6) failed in the 1987 run, this treatment was eliminated in the 1988 run. The simulated fermentation broth treatment was used only on the 1988 run. Because of the limited amount of fermentation

broth, this treatment was used only on 1988 samples having a moisture content of 26.8%. The specifications of the treatments are shown in Table 11.

### 3.2.1. Application levels

Each treatment was applied to the moist corn at the level of 0.5 and 1.0% propionate based on the weight of corn (as-is basis).

TABLE 11. Treatments and Their Specifications

| Treatments | pH | Propionate Content (%) | Water Content (%) | Application Level Propionate g/100g Corn |
|---|---|---|---|---|
| Propionic Acid | 1.70 | 97.67 | 0.00 | 1.00 |
| Propionic Acid | 1.70 | 97.67 | 0.00 | 0.50 |
| Salt Solution | 9.60 | 31.19 | 50.01 | 1.00 |
| Salt Solution | 9.60 | 31.19 | 50.01 | 0.50 |
| Semi Acidified | 4.86 | 21.38 | 57.79 | 1.00 |
| Semi Acidified | 4.86 | 21.03 | 58.20 | 0.50 |
| Acidified | 1.70 | 18.01 | 59.82 | 1.00 |
| Acidified | 1.70 | 18.04 | 59.92 | 0.50 |
| Sim. Fer. Brth. | 3.70 | 40.00 | 33.85 | 1.00 |
| Sim. Fer. Brth. | 3.70 | 40.00 | 33.85 | 0.50 |
| Ferment. Broth | 4.10 | 28.09 | 28.59 | 1.00 |
| Ferment. Broth | 4.10 | 28.09 | 28.59 | 0.50 |

## 3.3. Preparation of Chemical Solutions

Chemical solutions were applied to 2100 g of moist corn unless otherwise indicated. Solutions were prepared as follows.

### 3.3.1. Propionic acid

Exactly 10.75 g and 21.50 g of 99% commercial propionic acid (Kemin Chemical Company) were added to the corn to obtain 0.5 and 1.0% propionate levels respectively.

### 3.3.2. Salt solution

Sodium propionate (Aldrich Chemical Company) and sodium acetate (Fisher Scientific Company, FSCO) were dissolved in a minumum amount of water at 25°C. Therefore moisture addition to the corn was minimized. The formula used to make this solution is shown Table 12.

TABLE 12. Contents of Salt Solutions for 2100 g of Corn

| Treatment | Application levels (g/100g corn) | pH | Sodium Propionate (g) | Sodium Acetate (g) | Water (g) | Total (g) |
|---|---|---|---|---|---|---|
| Salt Solution | 0.50 | 9.60 | 13.95 | 2.88 | 16.84 | 33.67 |
| Salt Solution | 1.00 | 9.60 | 27.90 | 5.76 | 33.68 | 67.34 |

### 3.3.3. Semi-acidified salt solution

The same salt solutions as above were prepared. Then concentrated HCl was added to bring the pH from 9.6 to 4.86. HCl addition caused some NaCl formation, therefore a sufficient amount of water was added to dissolve this formed salt. Table 13 shows the amounts of HCl and water added to the salt solutions.

TABLE 13. HCl and Water Amounts for Semiacidification of Salt Solutions

| Treatments | Application Levels (g/100g corn) | pH | HCl (g) | Water (g) | Total (g) |
|---|---|---|---|---|---|
| Semi Acidified | 0.50 | 4.86 | 11.07 | 5.19 | 49.93 |
| Semi Acidified | 1.00 | 4.86 | 21.21 | 9.60 | 98.15 |

### 3.3.4. Acidified salt solution

The same formula of salt solutions was used at both application levels. This time, HCl was used to adjust the pH of the solutions to 1.7. Then some water was added to dissolve formed NaCl. They are shown in Table 14.

TABLE 14. HCl and Water Amounts to Acidify Salt Solutions

| Treatments | Application Levels (g/100g Corn) | pH | HCl (g) | Water (g) | Total (g) |
|---|---|---|---|---|---|
| Acidified | 0.50 | 1.70 | 17.85 | 6.70 | 58.22 |
| Acidified | 1.00 | 1.70 | 36.11 | 13.20 | 116.65 |

3.3.5. Simulated fermentation broth

Starting broth composition and a 20-time concentrated simulated broth composition were obtained from Dr. Glatz in our department.

Typical starting broth composition was as follows

| | |
|---|---|
| Trypticase peptone (BBL) | 5.00 g/l |
| Yeast Extract (Difco) | 5.00 g/l |
| Glucose (FSCO) | 40.00 g/l |
| NaCl (FSCO) | 1.67 g/l |
| $K_2HPO_4$ (FSCO) | 0.83 g/l |

At the end of the fermentation, it was assumed that there would be 2% propionate in the broth. The glucose would be almost entirely used up. The trypticase and yeast extract would be about half gone, and the salts might be relatively unchanged. To increase propionate amount from 2% to 40% for corn treatment, a 20-time concentration of fermentation broth was needed. A 20-time concentrated simulated broth would contain:

| | |
|---|---|
| Trypticase peptone (BBL) | 50.0 g/l |
| Yeast extract (Difco) | 50.0 g/l |
| Glucose (FSCO) | 20.0 g/l |
| NaCl (FSCO) | 33.0 g/l |
| $K_2HPO_4$ (FSCO) | 17.0 g/l |
| Propionic acid (Kemin) | 409.6 g/l |
| Acetic acid (FSCO) | 81.9 g/l |

About 26.25 g and 52.5 g of this mixture were added to the moist corn to obtain 0.5 and 1.0% application doses, respectively.

3.3.6. Fermentation broth

Fermentation broth (350 ml) was obtained from Anthony Weier (1989), a graduate student, in our department. Fermentation with Propionibacterium thoenii strain P9 was run in batch mode for 5-7 days with pH controlled by the addition of NaOH. The initial medium composition (Weier, 1989) is given Table 15. Propionate concentration in the broth was 0.94%. Then the broth was evaporated at 63 $^{o}$C. After evaporation, propionate concentration in the solids part reached to 4.84%. The ratio of acetate to propionate was 0.92 in the evaporated broth. To increase propionate concentration up to 25%, 15.34 g of propionic acid and 14.14 g of acetic acid were added. There were some solids in the broth, therefore 16.84 g of water were added to dissolve

most of them. Since some solids still remained in the broth, then a mixture of propionic acid, acetic acid and water was prepared to dissolve the solids. In order to maintain the original ratio of acetate to propionate in the evaporated broth, the ratio of propionic acid:acetic acid:water was 100:92:100 in the mixture. About 127 g of this mixture were sufficient to dissolve the solids. Final pH of the broth was 4.1.

About 32.04 g and 64.08 g of this final mixture were added to 1800 g of moist corn to reach 0.5 and 1.0% propionate application levels, respectively.

TABLE 15. Initial Composition of Fermentation Broth (Weier, 1989)

| Component | Amount Per Liter |
|---|---|
| Yeast Extract[a] | 6.00 g |
| Trypticase Peptone[b] | 2.87 g |
| Sodium Lactate 60% Syrup[c] | 30.00 ml |
| NaCl | 1.67 g |
| $K_2HPO_4$ | 0.83 g |

[a]Difco Laboratories, Detroit, MI.

[b]Baltimore Biological Laboratories Inc. (BBL), Cockeysville, MD.

[c]Fisher Scientific Co. (FSCO), Chicago, IL.

## 3.4. Treatment of Corn

About 2100 g of cleaned corn was weighed and transferred into a Dayton Shaded Pole (Gearmotor) rotator model 3M328A (Page 43). Mixing was improved by adding an iron rod at the bottom site of the rotator. Appropriate amounts of solutions were added to the corn by using a syringe while the corn was being mixed. Then mixing was allowed to continue for 10 minutes.

Each treatment was replicated three times, with each replicate consisting of one 2100-g bag of corn at each moisture content. Because of the limited amount of fermentation broth, 1800 g of corn were used in this treatment. Moisture contents of the samples after treatment are given in Table 16.

## 3.5. Determination of Moisture Content

Initial moisture content of the corn and the treated corn were determined by drying about 10 g of whole corn at 103 $^{o}$C for 72 h. The moisture content wet basis was found by using the following formula:

$$MCWB = (W_1 - W_2)100 / W_1$$

Where

MCWB = Moisture content wet basis (%)

$W_1$ = Initial weight (g) of the corn

$W_2$ = Final weight (g) of the corn after being in the oven for 72 hours.

FIGURE 5. Rotator

TABLE 16. Moisture Contents of the Samples After Treatment (Means of Three Replicates)

| | Harvesting Moistures (%) | | | | |
|---|---|---|---|---|---|
| | 1987 Samples | | | 1988 Samples | |
| Treatments | 26.8 | 23.0 | 17.6 | 26.8 | 29.6 |
| Propionic Acid | | | | | |
| 0.5% | 27.12 | 23.49 | 18.50 | 27.20 | 29.56 |
| 1.0% | 27.20 | 23.45 | 18.68 | 27.08 | 29.79 |
| Salt Solution | | | | | |
| 0.5% | 27.17 | 23.41 | 18.34 | _ | _ |
| 1.0% | 27.34 | 23.30 | 18.45 | _ | _ |
| Semi Acidified Salt Solution | | | | | |
| 0.5% | 27.16 | 23.59 | 19.82 | 26.75 | 29.22 |
| 1.0% | 27.80 | 24.54 | 20.05 | 27.44 | 29.79 |
| Acidified Salt Solution | | | | | |
| 0.5% | 27.10 | 23.92 | 19.14 | 27.03 | 29.87 |
| 1.0% | 27.75 | 24.73 | 20.23 | 27.78 | 30.28 |
| Simulated Fermentation Broth | | | | | |
| 0.5% | _ | _ | _ | 26.88 | 29.49 |
| 1.0% | _ | _ | _ | 27.43 | 29.66 |
| Fermentation Broth | | | | | |
| 0.5% | _ | _ | _ | 27.25 | _ |
| 1.0% | _ | _ | _ | 27.42 | _ |

## 3.6. Storage of Corn

Samples Harvested in 1987:

Treated and untreated corn were held in polyethylene bags (0.0018 in. thickness) at approximately 23 $^{o}$C for 10 months period of time. Since humidity in the storage room was not controlled, the samples gradually lost some moisture during this time.

At the end of the 36th week, examination of the samples at initial moisture contents of 17.6% and 23.0% was stopped. At the end of the 10th month, the moisture contents of samples having an initial moisture content of 26.8% were increased to 26%. The water amount added into samples was calculated as follows;

$$W_f - W_0 = ( 1 / (100 - M_f) ) (M_f - M_0) (W_0)$$

where

$M_f$ = Final moisture content, % wet basis

$M_0$ = Initial moisture content, % wet basis

$W_0$ = Initial weight

$W_f$ = Final weight

After two months of storage at the above conditions, another moisture adjustment was applied to the samples. Then samples were transferred to a moisture chamber in double bags each having a thickness of 0.0018 in.

Temperature and relative humidity in the chamber were maintained at 25∓2 $^{o}$C and 75-95% respectively.

At the end of 84th week the samples were transferred to 1-quart mason jars covered by cheese cloth.

Samples Harvested in 1988:

Treated and untreated samples were transferred to Ziploc freezer bags (10.56 in x 11 in x 2.7 mils), sealed and stored in the moisture chamber with 75 to 95% relative humidity and 25∓2 $^{o}$C. Air was being circulated in the chamber during storage.

At the end of 32 weeks samples were transferred to 1-quart mason jars covered with cheese cloth to increase air available to the samples.

## 3.7. Sampling

### 3.7.1. Interval

The samples were observed visually during storage for signs of mold growth and tested every fifteen days for the presence of internal fungi and percentage fungal colonization for the first three months. After three months the corn was tested monthly for the rest of the storage time. Weekly samples were collected from untreated samples.

3.7.2. Determination of color of samples

Colors of samples were determined using a Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia). Before measuring color, the colorimeter was calibrated (zero scale calibration and standardization on the white calibrated standard) and daylight was chosen as the light source. The sample in a container was placed in the sensing position and then the readings were recorded in 'Lab' values. In the 'Lab' unit;

L = Indicates lightness

a = Indicates red (+) and green (-).

b = Indicates yellow (+) and blue (-).

The greater the 'a' and 'b' numbers, the redder and the more yellow the samples. Analysis of variance for the results were done using the GLM Statistical Analysis System (SAS). Comparison of means for each combination of propionate treatment was based on Duncan's multiple range test.

3.7.3. The presence of fungi

Fifty kernels from each treatment were surface-sterilized for one minute in 1.0% sodium hypochlorite, rinsed in sterile distilled water and then placed, aseptically onto malt-salt agar (Christensen and Meronuck, 1986).

Each agar plate contained ten kernels and plates were incubated at 25°C for 7 days. On the of morphological characteristics, the percentage fungal colonization of the plated corn was determined.

Preparation of 1 L of malt-salt agar was done as follows:

Solution A: 20 g malt extract, and 20 g agar were dissolved in 500 ml of distilled water.

Solution B: 60 g NaCl dissolved in 500 ml of distilled water.

Solution A and B were autoclaved in separate flasks, cooled to 50°C, mixed and poured into plates.

3.7.4. Identification of the fungi

Fungi were grown in slide culture (Taschdjian, 1954). Sabouraud Dextrose Agar (SDA, BBL) was poured into petri dishes and solidified. A cover slip was grasped with flamed forceps and dipped in alcohol and flamed. The cover slip was cooled for a moment, then dipped into 0.75% SDA. The coated cover slip was placed on the surface of a petri dish containing solid culture medium. A pinpoint inoculum from plated moldy kernels was placed in the center of each coated cover slip for each type of mold. Plates were inverted and incubated for 3 days at room temperature. Then the cover slip was removed from the plate and inverted into a drop of

lactophenol-cotton blue on a slide. The slides were examined under a microscope and the fungi were identified according to their morphological characteristics (Raper and Fennell, 1977; Barnett and Hunter, 1987).

A pinpoint inoculum from plated moldy kernels were also examined in a drop of water. In this way a pinpoint inoculum was removed from plated moldy kernel with a sterilized needle and dipped in a drop of water on a slide and covered with a cover slip and examined under a microscope.

3.7.5. Inoculation of the corn

The organisms used in this study were Aspergillus flavus and Penicillium spp. obtained from fresh corn. Surface sterilized corn kernels were plated on malt-salt agar and incubated at 25 $^{o}$C for 7 days. Aspergillus flavus and Penicillium spp. were isolated from plated moldy kernels and grown on SDA for 6 days at 25 $^{o}$C. Subcultures were grown in the same way to prepare spore suspensions. Spore suspensions were prepared by washing off the surface growth of subcultured agar plates with 0.1% peptone (Difco) dilution water. Appropriate serial dilutions were made by following standard methods and pour-plated with acidified Potato Dextrose Agar (PDA, Difco) for determining numbers of spores per ml.

The corn harvested with 26.8% moisture content in 1987 was inoculated four times. The first inoculation was applied at the end of one year of storage period with A. flavus and Penicillium spp. at the inoculation rates of $10^2$ spores/g corn (wet basis) and 10 spores/g corn respectively. In other inoculations only the A. flavus strain was used. A second, third, and fourth inoculation were applied on the same samples at 2 month intervals. Spore concentrations in these inoculations were $10^4$, $10^6$, and $10^5$ spores/g corn respectively.

The 1988 samples were inoculated with A. flavus spores after 6 months of storage period. Samples having initial moisture contents of 29.6% were inoculated two times with spore concentrations of $2.4 \times 10^5$ and $7.1 \times 10^5$ spores/g corn respectively. Samples at 26.8% moisture content were also inoculated at the rates of $1 \times 10^4$ spores/g corn and $4.8 \times 10^5$ spores/g corn. The second inoculation was applied after samples were transferred into mason jars.

Each time a untreated sample was also inoculated at the same spore inoculation concentration.

### 3.7.6. Determination of total aerobic plate count

Total count of microorganisms was determined in two replicates of every treatment by placing 11 g of the corn into 99 ml of 0.1% peptone water. Appropriate serial

dilutions were made by following standard methods (Messer et al., 1985) and were pour plated with All Purpose Tween agar (APT, FSCO). Plates were incubated at 32 °C for 50 h. All plates containing between 25 and 250 colonies were counted (Messer et al., 1985), and the average of duplicate counts were recorded. Analysis of variance was performed on the microbiological data after logarithmic transformation.

### 3.7.7. Determination of corn grade

Samples of corn from each treatment stored one and two years were graded according to the official United States standards for grain by the Grain Quality Laboratory, Iowa State University.

# 4. RESULTS AND DISCUSSION

The number of infected kernels changed from a few per cent in treated samples to 100% in untreated samples. The outward appearance of the grain did not give a good indication of the degree of internal infection at the beginning of deterioration. During the whole experiment, mold growth in the samples was checked by plating surface-sterilized samples on Malt Salt Agar medium. In the tests, grain was considered no longer mold-free when more than 10 per cent of the kernels became internally invaded by molds.

## 4.1. Corn

Natural microflora of corn harvested in two successive years were different. The percentage of moldy kernels and the percentage of fungus infected kernels are given in Tables 17 and 18 and Figures 6, 8, and 10. Among 1987 samples, zero-time control samples had over 80% infection by Fusarium. The corn at 26.8% and 23% moisture got moldy in the first week of storage. Penicillium and Fusarium were dominant. Despite the existence of Penicillium 11% and Aspergillus 9% in the corn at 17.6% moisture content, there were no visible moldy kernels in this sample at the end of two weeks. The visible mold growth occurred at the 6th week of storage period.

TABLE 17. The Percentage of plated Kernels Invaded by Various Fungi in Untreated Corn Harvested in 1987[a]

| Weeks Stored | Initial Moisture Content (%) | Plated Moldy Kernel (%) | Kernels Infected With (%) | | |
|---|---|---|---|---|---|
| | | | Aspergillus | Penicillium | Mucor and Fusarium |
| 0 | 26.8 | 99 | 3 | 3 | 79 |
| | 23.0 | 87 | 1 | 6 | 86 |
| | 17.6 | 87 | 3 | 9 | 89 |
| 1 | 26.8 | 100 | 1 | 50 | 100 |
| | 23.0 | 99 | 4 | 78 | 100 |
| | 17.6 | 98 | 0 | 0 | 98 |
| 2 | 26.8 | 100 | 1 | 69 | 99 |
| | 23.0 | 100 | 7 | 79 | 99 |
| | 17.6 | 100 | 9 | 11 | 99 |
| 3 | 26.8 | Discarded | | | |
| | 23.0 | Discarded | | | |
| | 17.6 | 100 | 2 | 15 | 99 |
| 6 | 17.6 | 100 | 5 | 51 | 97 |

[a]The numbers are the mean values of three replications.

In the 1988 samples, the dominant mold was A. niger. Later on, A. flavus and Penicillium growth were abundant. The numbers of broken kernels and insect parts were greater in 1988 samples than in 1987 samples.

## 4.2. Chemicals

The effectiveness of treatments is discussed in this section. Mold-free days of treatments are given in Tables 19 and 20 for both 1987 and 1988 samples.

TABLE 18. Percentages of Plated Kernels Invaded by Various Fungi in Untreated Corn Harvested in 1988[a]

| Weeks Stored | Initial Moisture Content (%) | Plated Moldy Kernel (%) | Kernels Infected With (%) 1[b] | 2[c] | 3[d] | 4[e] |
|---|---|---|---|---|---|---|
| 0 | 26.8 | 100 | 5 | 75 | 21 | 47 |
| | 29.6 | 100 | 22 | 97 | 6 | 3 |
| 1 | 26.8 | 100 | 14 | 76 | 65 | 33 |
| | 29.6 | 100 | 60 | 94 | 9 | 1 |

[a]The numbers are the mean values of three replications.

[b]Aspergillus flavus.

[c]Aspergillus niger.

[d]Penicillium.

[e]Cladosporium.

TABLE 19. Mold-Free Storage Time (Weeks) for Corn Harvested in 1987 With Various Moisture contents (MC) and Stored at 23-25°C[a]

| Treatments | 0.5% Application Rate | | | 1.0% Application Rate | | |
|---|---|---|---|---|---|---|
| | 17.6%MC | 23.0%MC | 26.8%MC | 17.6%MC | 23.0%MC | 26.8%MC |
| Control[b] | <1 | <1 | <1 | <1 | <1 | <1 |
| Salt Solution (pH 9.60) | 14 | 8 | <1 | 36+[c] | 36+ | 60 |
| Semi Acidified (pH 4.86) | 36+ | 36+ | 74 | 36+ | 36+ | 86+ |
| Acidified (pH 1.70) | 36+ | 36+ | 86+ | 36+ | 36+ | 86+ |
| Propionic Acid | 36+ | 36+ | 86[d]+ | 36+ | 36+ | 86[e]+ |

[a]Mean values of three replicates.

[b]Untreated corn.

[c]No mold growth was observed up to observation point.

[d]One replication of this treatment failed at the 81th week of the storage.

[e]One replication of this treatment failed at the 82th week of the storage.

TABLE 20. Mold-Free Storage Time (Weeks) for Corn Harvested in 1988 With 26.8 and 29.6% Moisture and Stored at 25°C[a]

| Treatments | 0.5% Application Rate | | 1% Application Rate | |
|---|---|---|---|---|
| | 26.8%MC | 29.6%MC | 26.8%MC | 29.6%MC |
| Control[b] | <1 | <1 | <1 | <1 |
| Semi Acidified (pH 4.86) | 42+[c] | 42+ | 42+ | 42+ |
| Acidified (pH 1.70) | 42+ | 42+ | 42+ | 42+ |
| Propionic Acid | 42[d]+ | 42+ | 42+ | 42+ |
| Simulation of Ferment. Broth (pH 3.40) | 42+ | 42+ | 42+ | 42+ |
| Fermentation broth (pH 4.10) | 42+ | - | 42+ | - |

[a]Mean values of three replicates.

[b]Untreated corn.

[c]No mold growth was observed up to observation point.

[d]One replication of this treatment failed at the 12th week of storage.

4.2.1. Salt solution (pH 9.6)

The salt solution was relatively slow in killing original fungi so that after treatment fungi were numerous, but in 2 weeks they were either very scarce or very abundant depending on whether the treatment rate was sufficient or not. Figures 7, 9, 11 and Tables 21, 22, and 23 show the effects of salt solution on corn with different moisture contents. The mold-killing effect of salt solutions was slowest on corn with low moisture content. Although 0.5% application rate was not enough to prevent the corn from spoilage, it retarded abundant mold growth for a short time period at the medium and low moisture contents. The principal fungi which grew in grain without adequate treatment were Aspergillus spp., Fusarium spp., and yeasts at 26.8%MC. The same fungi plus Penicillium spp. grew at 23.0%MC. Aspergillus spp. and Penicillium spp. were found at 17.6% MC. The 1% application rate was sufficient to preserve corn with 26.8% MC for a long time. These results show good agreement with Sauers and Burroughs (1974). The second inoculation of the corn at 26.8% with A. flavus spores caused failure of the 1.0% salt treatment (pH 9.6) after 60 weeks of storage.

Therefore, it was concluded that the natural salt solution treatment (pH 9.6) even at the high application

level likely will not prevent corn from deterioration if any further contamination occurred in the storage. This treatment also had the most abundant yeast growth among all treatments.

4.2.2. Semi-acidified and acidified salt solutions

The inhibition effect of sodium propionate solutions increased with decreasing pH of the solutions. This result was in agreement with the literature (Kirby et al., 1937; Wyss et al., 1945; Simon and Beevers, 1951; Hesseltine, 1952; Samson et al., 1955; Fencl and Leopold, 1957; Eckert, 1967). There are several possible interpretations of this finding (1) Antifungal activity of organic acids is the highest in their undissociated forms. The carboxylic acids (benzoic, propionic and sorbic) have pKa values in the range 4-5, therefore, their activity is greatest below pH 4 and is greatly decreased at pH values of 6 or higher. At a pH of 4.00, 88% of the propionate is undissociated while at a pH of 6.0, only 6.7% remains undissociated (Jay, 1978). (2) The charge of certain components of the cell membrane increases with decreasing pH and thus the binding of fatty anions to protein occurs more readily. (3) The undissociated form of organic acids may penetrate more easily, and once within the cell is largely neutralized to the salt which might then act as the inhibitory form.

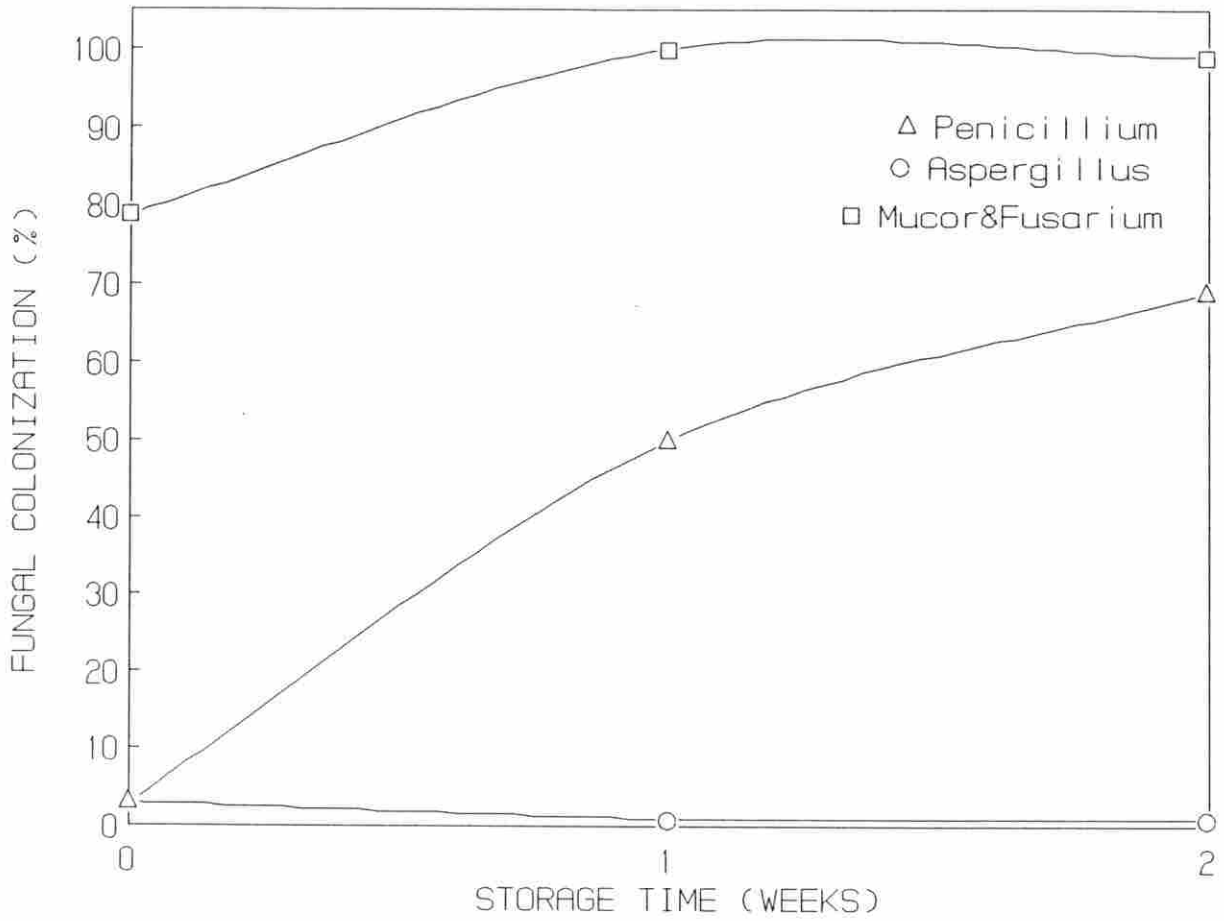



FIGURE 6. Fungal Colonization in Untreated Corn Harvested in 1987 with 26.8% Moisture

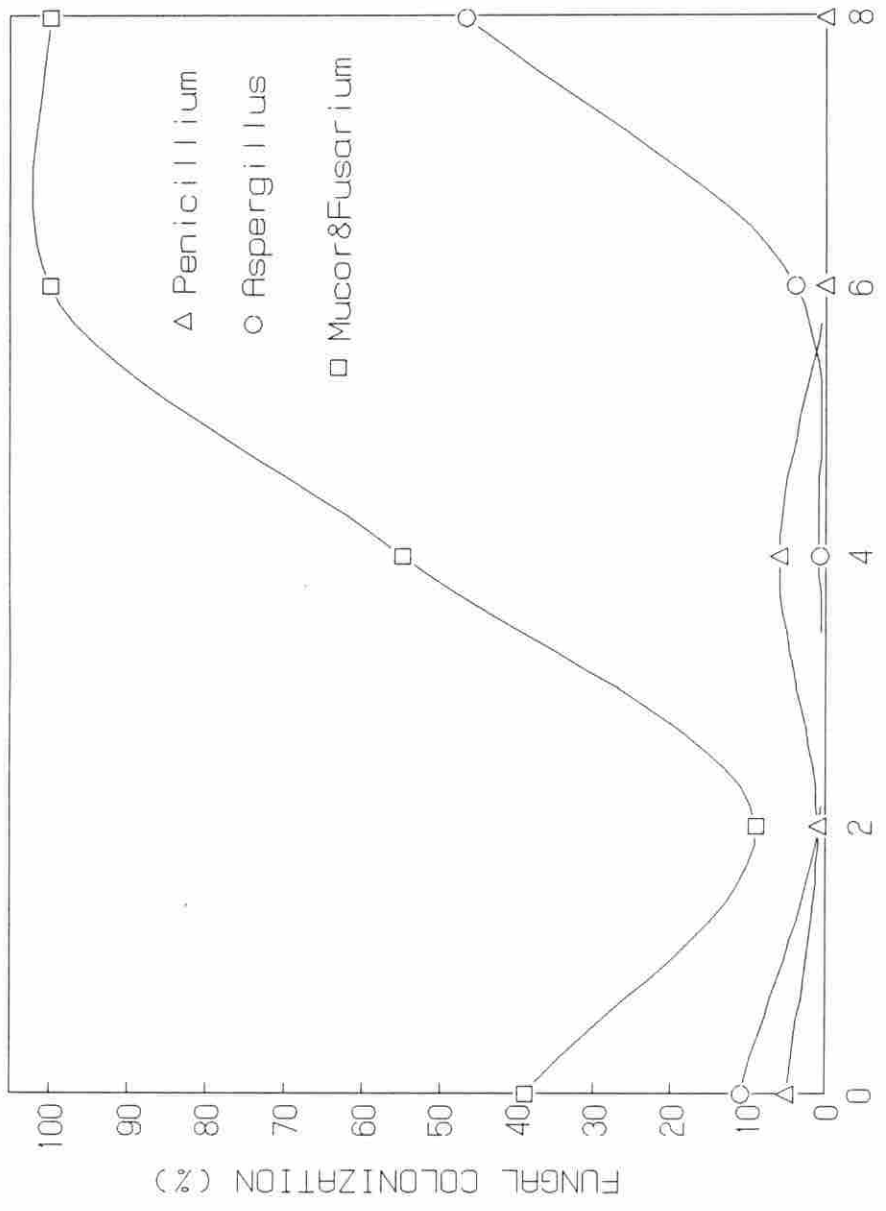



FIGURE 7. Fungal Colonization in Corn Harvested in 1987 with 26.8% Moisture and Treated With 0.5% Sodium Propionate (pH 9.6)

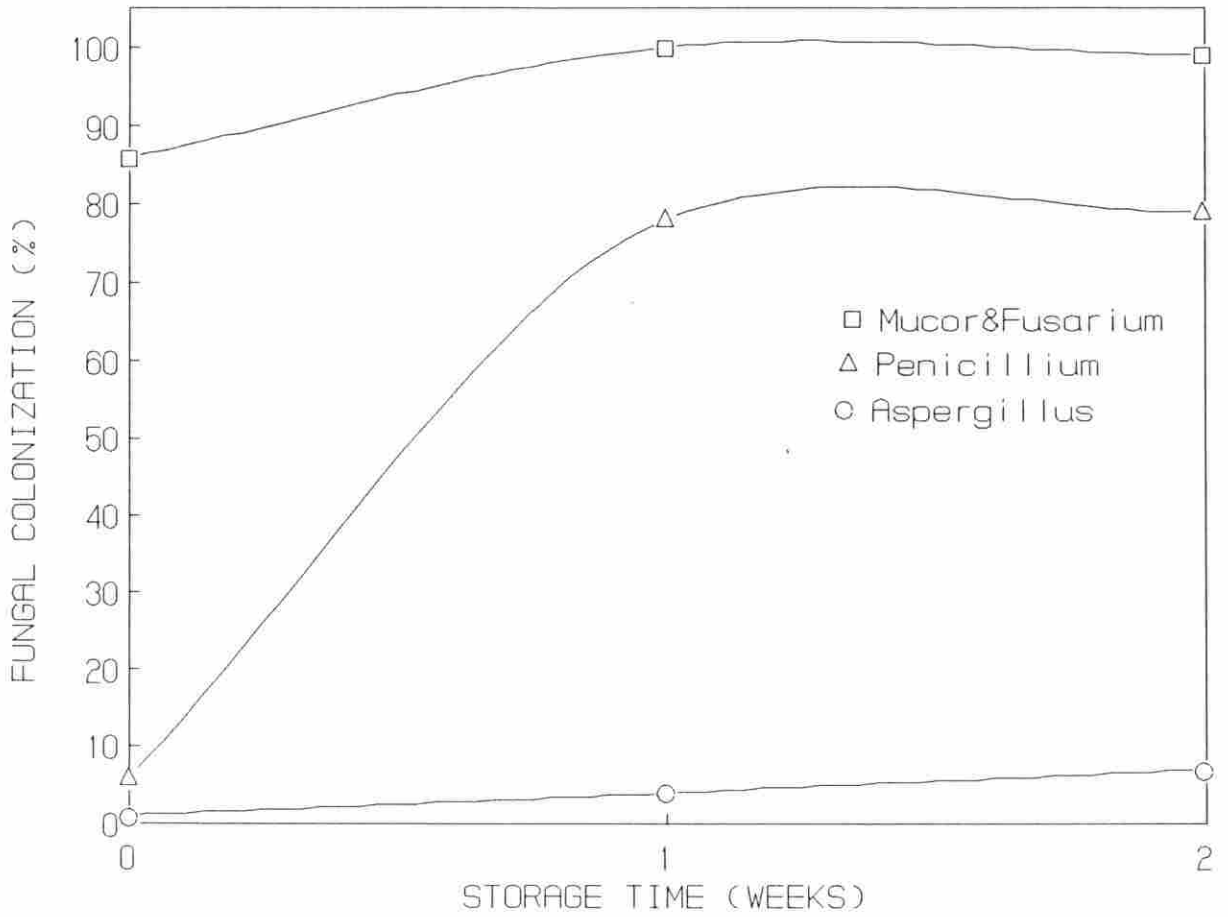



FIGURE 8. Fungal Colonization in Untreated Corn Harvested in 1987 with 23% Moisture

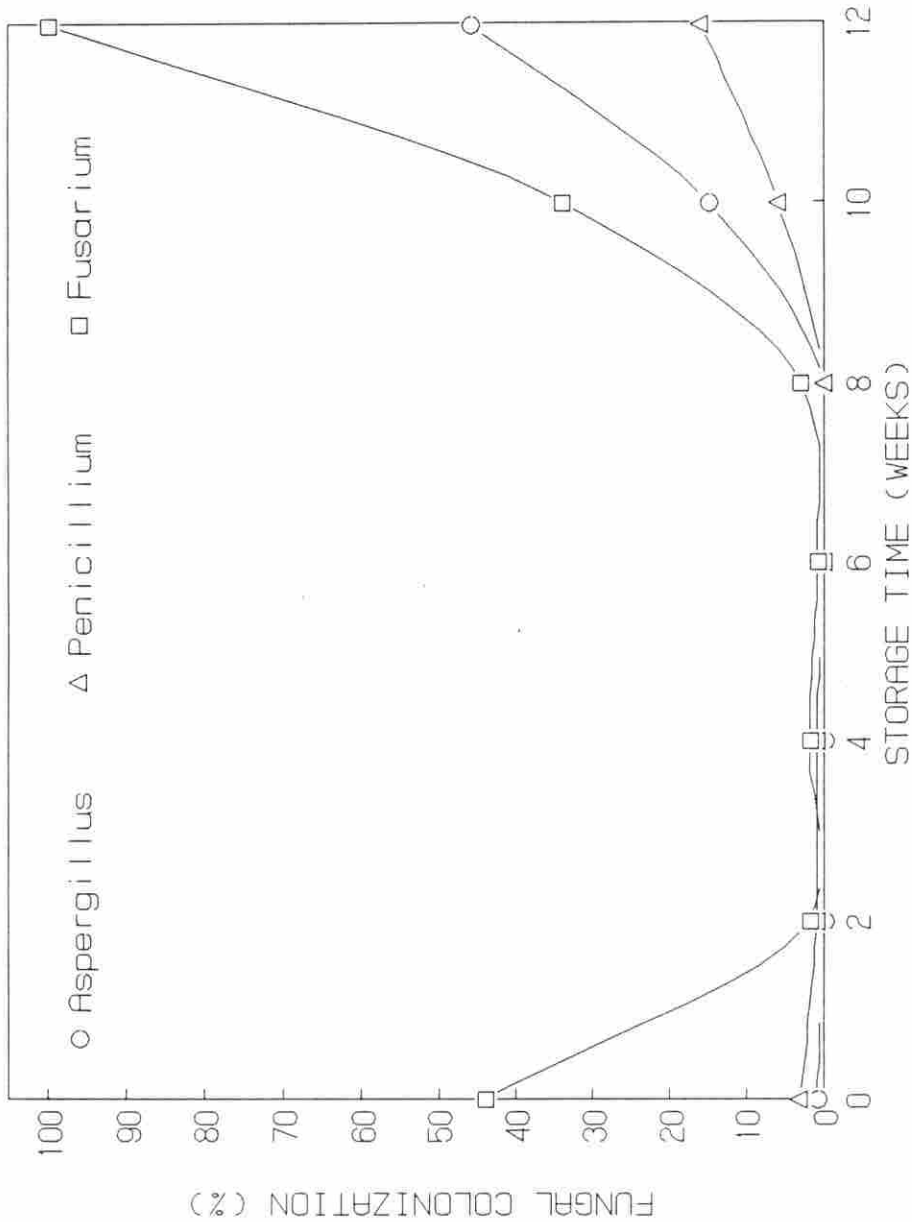



FIGURE 9. Fungal Colonization in Corn Harvested in 1987 with 23% Moisture and Treated with 0.5% Sodium Propionate (pH 9.6)

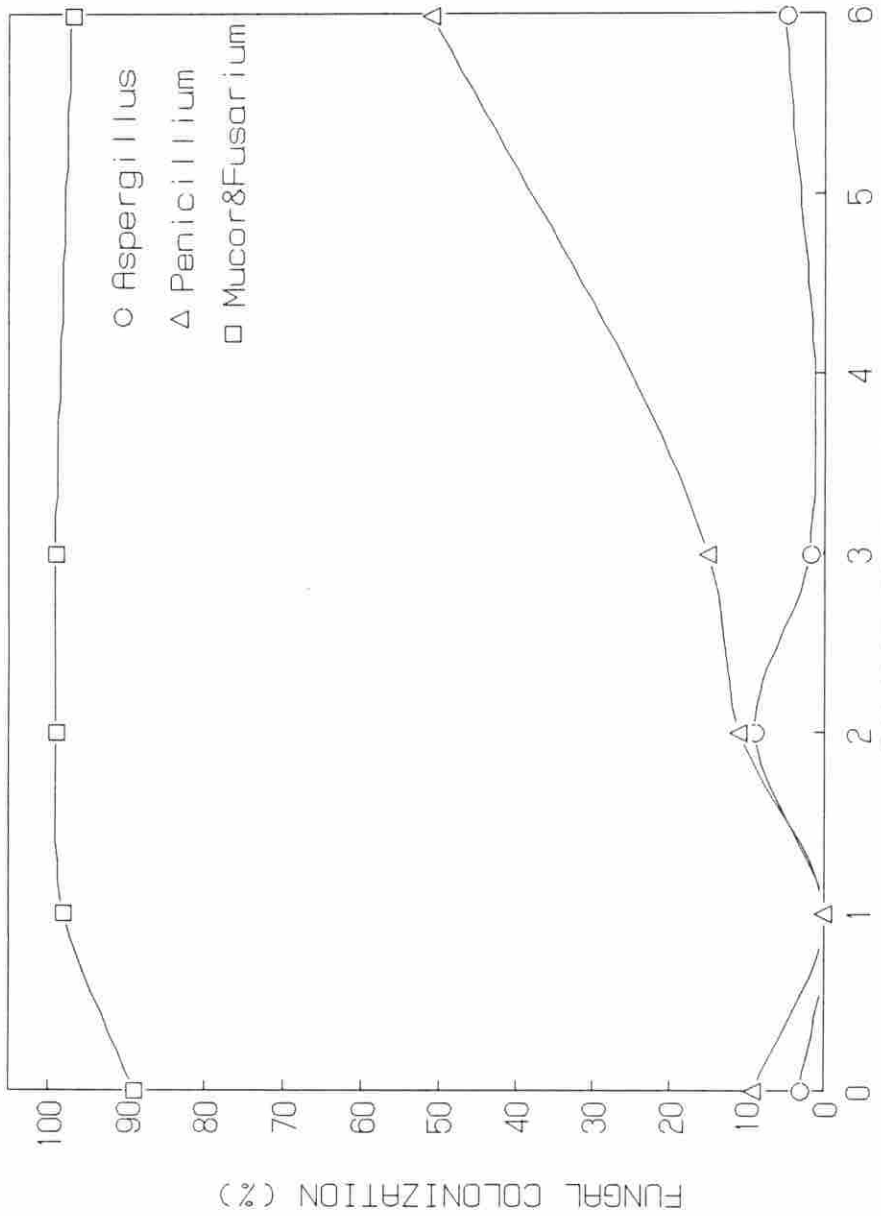



FIGURE 10. Fungal Colonization in Untreated Corn Harvested in 1987 with 17.6% Moisture

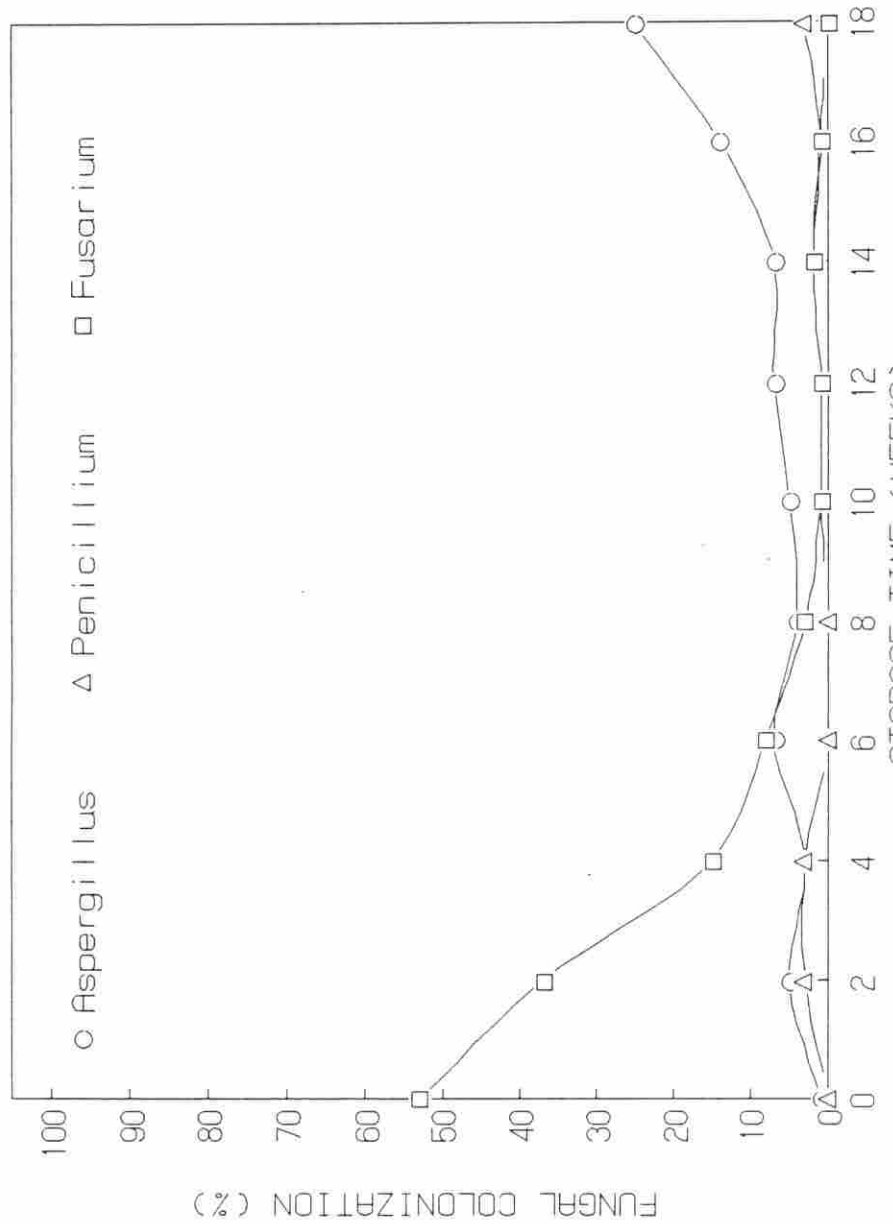



FIGURE 11. Fungal Colonization in Corn Harvested in 1987 with 17.6% Moisture and Treated with 0.5% Sodium Propionate (pH 9.6)

TABLE 21. Percentages of Plated Kernels Infected With Fungi in Corn Harvested at 26.8% Moisture and Treated With Sodium Propionate Solution, pH 9.6[a]

| Weeks Stored | Moisture Content (%) | Plated Moldy Kernel (%) | Kernels Infected With (%) | | | |
|---|---|---|---|---|---|---|
| | | | Aspergillus | Penicillium | Fusarium and Mucor | Yeast |
| | | | 0.5% Application Rate | | | |
| 0 | 26.80 | 53 | 11 | 5 | 39 | 0 |
| 2 | | 10 | 1 | 1 | 9 | 0 |
| 4 | | 55 | 1 | 6 | 55 | 45 |
| 6 | | 100 | 4 | 0 | 100 | 13 |
| 8 | 28.34 | 100 | 47 | 0 | 100 | 0 |
| | | | 1.0% Application Rate | | | |
| 0 | 26.80 | 43 | 3 | 9 | 32 | 0 |
| 2 | | 1 | 0 | 1 | 1 | 0 |
| 4 | | 0 | 0 | 0 | 0 | 0 |
| 60 | | 100 | 100 | 0 | 0 | 10 |

[a]Means of three replicates.

TABLE 22. Percentages of Plated Kernels Infected With Fungi in Corn Harvested at 23.0% Moisture and Treated With Sodium Propionate Solution, pH 9.6[a]

| Weeks Stored | Moisture Content (%) | Plated Moldy Kernel (%) | Kernels Infected With (%) | | | |
|---|---|---|---|---|---|---|
| | | | Aspergillus | Penicillium | Fusarium | Yeasts |
| | | | 0.5% Application Rate | | | |
| 0 | 23.00 | 48 | 1 | 3 | 44 | 0 |
| 2 | | 3 | 0 | 1 | 2 | |
| 4 | | 2 | 0 | 1 | 2 | 0 |
| 6 | | 1 | 0 | 0 | 1 | 0 |
| 8 | | 3 | 0 | 0 | 3 | 11 |
| 10 | 20.13 | 34 | 15 | 6 | 34 | 2 |
| 12 | | 100 | 46 | 16 | 100 | 0 |
| | | | 1.0% Application Rate | | | |
| 0 | | 44 | 2 | 2 | 43 | 0 |
| 2 | | 1 | 0 | 1 | 1 | 0 |
| 4 | | 0 | 0 | 0 | 0 | 0 |
| 36 | | 0 | 0 | 0 | 0 | 0 |

[a]Means of three replicates.

TABLE 23. Percentages of Plated Kernels Infected With Fungi in Corn Harvested at 17.6% Moisture and Treated With Sodium Propionate Solution, pH 9.6[a]

| Weeks Stored | Moisture Content (%) | Plated Moldy Kernel (%) | Kernels Infected With (%) | | |
|---|---|---|---|---|---|
| | | | Aspergillus | Penicillium | Fusarium |
| | | | 0.5% Application Rate | | |
| 0 | 17.60 | 53 | 1 | 0 | 53 |
| 2 | | 38 | 5 | 3 | 37 |
| 4 | | 15 | 3 | 3 | 15 |
| 6 | | 9 | 7 | 0 | 8 |
| 8 | | 7 | 4 | 0 | 3 |
| 10 | | 5 | 5 | 1 | 1 |
| 12 | | 7 | 7 | 1 | 1 |
| 18 | 15.49 | 27 | 25 | 3 | 0 |
| | | | 1.0% Application Rate | | |
| 0 | 17.60 | 42 | 1 | 0 | 42 |
| 2 | | 11 | 2 | 0 | 9 |
| 4 | | 3 | 0 | 0 | 3 |
| 6 | | 3 | 1 | 1 | 2 |
| 8 | | 3 | 3 | 0 | 1 |
| 36 | | 0 | 0 | 0 | 0 |

[a]Means of three replicates.

The first-year samples at 26.8% MC treated with 0.5% semi-acidified salt solution failed after the 4th inoculation. They were mold-free for 74 weeks of the storage when the fourth inoculation was applied.

Despite four inoculations of the first-year samples treated with semi-acidified salt solution at the level of 1% and acidified salt solution at the rate of 0.5% and 1.0%, they were still mold-free for 86 weeks when the experiment was stopped. Although two inoculations were applied to the samples harvested in 1988, they were also mold-free for 42 weeks when the experiment was stopped. These results show that semi-acidified or acidified propionate and acetate salts prufied from propionic acid fermentation can be succesfully used as grain preservative.

Simon and Beevers (1951) stated that the effect of a given pH change depended on the pKa value of the compound. For pH levels below the pKa level, the pH changes affected the antifungal activity very little but as the pH was increased above pKa, the effect of the organic acid decreased by as much as three times for each pH unit. They recommended a pH value two or more pH units below the pKa value of a compound. Kirby at al. (1937) stated that molds have a very wide growth optimum with respect to hydrogen ion concentration. They found that at high pH values (5.5 to

6.0) acetic acid retarded initial growth of A. niger and Rhizopus nigricans and had only a slight effect on the ultimate growth of these molds.

Bandelin (1958) investigated the efficacy of some compounds having antifungal activity at pH levels of 3, 5, 7 and 9 against A. niger, Penicillium citrinum, Alternaria solani and Chactomium globosum. He reported that antifungal activity of investigated compounds progressively decreased with increasing pH values to the point that some were totally ineffective in the neutral and alkaline range. He showed that the activity of propionic acid was quite high at pH 3.0, fair at pH 5.0, greatly decreased at pH 7.0, and failed totally at pH 9.0.

The antifungal efficiency of propionic acid at different pH's in dextrose-nutrient broth on A. niger was investigated by Wyss et al. (1945). It was found that the inhibiting concentration of propionic acid showed an increase with increasing pH values, namely, 0.1% at pH 4.5, 0.3% at pH 5.5, 2.0% at pH 6.5, 5.0% at pH 7.5 were necessary. Strider and Winstead (1960) showed that 0.5% acetic acid was toxic to Cladosporium cucumerinum at pH 5.0 but not at 6.0 and that 1.5% acetic acid was toxic at both pH values.

4.2.3. Simulation of fermentation broth, fermentation broth and propionic acid

All treatments showed fungicide effects, totally killed natural microflora of the corn, and provided safe storage for long time periods. Failure occurred after 81 weeks of storage in only one replication of the 0.5% and 1.0% application levels of pure propionic acid in the first year samples having a moisture content of 26.8%. Among 1988 samples, only one replication of 0.5% pure propionic acid-treated corn with the moisture content of 26.8% failed. A. flavus caused the failure of these samples. Since deterioration occurred in only one replication while the other two replications were still mold free, this result was not sufficiently clear to evaluate the treatment.

No mold growth was observed in corn treated with simulated fermentation and propionic acid fermentation broths treated corn. This result interesting to show that even unpurified fermentation broth can be used as grain preservative for long term storage period.

## 4.3. Comparisons of Media Used for Checking Mold Spores After Inoculation of the Corn

After inoculation of the samples with A.flavus, PDA, All Purpose Tween (APT) and MSA were used to follow the growth of inoculated mold spores (Page 72). Therefore,

surface unsterilized samples taken after 16 h, 32 h, 82 h, and 10 days of inoculation and surface sterilized samples taken after 15 days of inoculation were plated on these media. The results showed that: APT provided the most favorable condition for growth of surface mold and bacteria; Mold and yeast growth were more abundant on PDA than on MSA; Surface sterilization killed almost all mold on the kernel surface; Inoculated spores were survived on the kernel surface for sometime but penetration into the corn was inhibited by chemicals; and MSA was a good medium to check internal mold growth of the corn. Once the mold growth occurred on the MSA, visible mold growth was occurred on the samples in the following weeks.

## 4.4 Color Evaluation

Tables 25 and 26 show the Hunter colorimeter 'Lab' values at various storage times. Treatments bleached the corn color and bleaching increased with increasing storage time. During storage air-dried corn (the same untreated corn was dried to 12% moisture content using a forced-air drier at room temperature for about 24 h) retained better color than propionate- treated corn. Air-dried corn had the highest 'L', 'a' and 'b' values which show

TABLE 24. Production of Mold After Inoculation of Corn Having 26.8%Moisture and Stored at 25°C and 75-90% RH[a]

| | Mold Growth on Plated Kernels (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16 h[b] | | | 32 h[b] | | | 82 h[b] | | | 10 days[b] | | | 15 days[c] | | |
| Treatment[d] | APT | PDA | MSA | APT | PDA | MSA | APT | PDA | MSA | APT | PDA | MSA | APT | PDA | MSA |
| SAS | | | | | | | | | | | | | | | |
| 0.5% | 95 | 93 | 51 | 92 | 83 | 14 | 63 | 48 | 2 | 41 | 31 | 1 | 0 | 0 | 0 |
| 1.0% | 100 | 51 | 0 | 99 | 71 | 1 | 100 | 31 | 0 | 93 | 26 | 0 | 0 | 1 | 0 |
| AS | | | | | | | | | | | | | | | |
| 0.5% | 100 | 96 | 86 | 100 | 62 | 15 | 100 | 97 | 0 | 83 | 58 | 2 | 0 | 0 | 0 |
| 1.0% | 95 | 29 | 0 | 100 | 71 | 0 | 80 | 25 | 0 | 30 | 5 | 0 | 3 | 0 | 0 |
| SFB | | | | | | | | | | | | | | | |
| 0.5% | 100 | 72 | 49 | 100 | 95 | 1 | 97 | 80 | 1 | 34 | 15 | 0 | 0 | 0 | 0 |
| 1.0% | 81 | 21 | 0 | 90 | 16 | 0 | 81 | 28 | 0 | 47 | 3 | 0 | 4 | 0 | 0 |
| PA | | | | | | | | | | | | | | | |
| 0.5% | 97 | 92 | 46 | 100 | 100 | 17 | 100 | 100 | 26 | 88 | 68 | 2 | 2 | 0 | 0 |
| 1.0% | 96 | 17 | 0 | 100 | 59 | 0 | 100 | 84 | 0 | 82 | 25 | 0 | 3 | 0 | 0 |
| FB | | | | | | | | | | | | | | | |
| 0.5% | 92 | 36 | 5 | 90 | 49 | 0 | 100 | 92 | 0 | 86 | 29 | 2 | 0 | 0 | 0 |
| 1.0% | 58 | 7 | 0 | 63 | 4 | 0 | 70 | 10 | 0 | 63 | 0 | 0 | 3 | 0 | 0 |

[a]Samples were inoculated with A. flavus at the rate of $4.8 \times 10^5$ spores/g corn and maintained in mason jars covered with cheesee cloth.

[b]Surface sterilization was not applied.

[c]Surface sterilization was applied.

[d]SAS is semi-acidified salts, AS is acidified salts, SFB is simulated fermentation broth, PA is propionic acid, FB is fermentation broth.

that air-dried corn was brighter, more red, and more yellow than propionate-treated corn. Among treatments, propionic acid treated corn had the highest 'L', 'a' and 'b' values followed by acidified salts-treated and semi-acidified salts-treated corn during the 8 months of the storage time. This shows that propionic acid treatment maintained corn color better than other treatments. Natural salt treatment gave the worst color. Figure 12 also shows the color differences among treatments.

The values taken after 4th, 8th and 24th months of storage time showed that 'L', 'a' and 'b' values decreased with increasing storage time. After 24 months of storage, there were no significant differences among treatments in terms of 'L', 'a' and 'b' values, but air-dried corn was significantly different from propionate-treated corn.

The color measurement on 1988 samples was made after 12 months of storage period. The 'L', 'a' and 'b' values were not significantly different among treatements on corn having moisture content of 26.8% (Table 27).

The treatments on corn with 29.6% moisture content gave different results (Table 28). Propionic acid treated corn had the highest 'L' value followed by acidified salt, simulation of fermentation broth and semi-acidified salts-treated corn. Semi-acidified salts-treated corn had slightly more red and yellow color than other treatments.

TABLE 25. Effects of Propionate Treatments on Color of Corn Harvested in 1987 and Stored for Four and Eight Months (Wang, 1989)

| Treatments | Storage Time (months) | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| 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 | $57.3^{bc}$ | $12.8^{b}$ | $24.9^{c}$ |
| Neutral Salt Solution | 4 | $48.2^{e}$ | $13.6^{ab}$ | $24.7^{c}$ |
| | 8 | $46.7^{e}$ | $13.3^{b}$ | $23.1^{d}$ |
| Semi-Acidified Salt Sol. | 4 | $55.7^{cd}$ | $12.3^{b}$ | $26.2^{b}$ |
| | 8 | $54.0^{d}$ | $12.0^{b}$ | $24.9^{c}$ |
| Acidified Salt Solution | 4 | $55.1^{cd}$ | $11.8^{b}$ | $26.4^{b}$ |
| | 8 | $53.5^{d}$ | $12.5^{b}$ | $25.3^{cb}$ |

$^{abcde}$Means with common superscripts in the same column are not significantly different at the 5% level.

The color quality differences can be also seen in Figures 13 and 14.

## 4.5. Total Aerobic Counts

Table 29 gives logarithmic transformations of total aerobic counts of 1988 and 1987 samples stored for one and

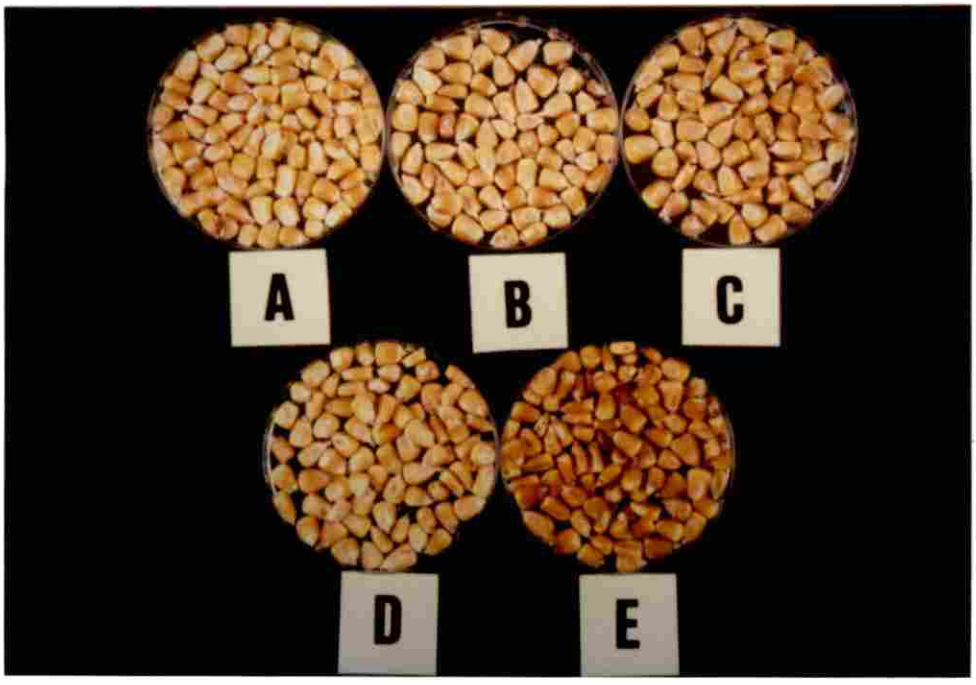
TABLE 26. Effects of Propionate Treatments on Color of Corn Harvested in 1987 and Stored for 24 Months

| Treatments | Application Level % | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| 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 | $33.5^{b}$ | $11.7^{b}$ | $16.1^{bc}$ |
| Semi-Acidified salt | 1.0 | $35.3^{b}$ | $9.5^{b}$ | $11.6^{c}$ |
| Acidified Salt | 1.0 | $33.3^{b}$ | $11.4^{b}$ | $15.6^{bc}$ |
| | 0.5 | $36.9^{b}$ | $11.2^{b}$ | $16.9^{b}$ |

$^{abc}$Means with common superscripts in the same column are not significantly different at the 5% level.

two years respectively. APT was used as medium and plates were incubated at 32 $^{o}$C for 50 h. Microorganisms grown on the plates were not identified.

The treatment effects on total counts of 1987 samples were not significant ($P>0.05$) and the mean values of total counts found on air-dried corn and propionate-treated corn were not significantly different. In 1988 samples, statistical analysis showed that the treatment effects on corn having initial moisture content of 29.6% were significant ($P<0.05$) and propionic acid-treated corn had




A: Air-dried corn
B: Propionic acid treated corn
C: Semi-acidified salts-treated corn
D: Acidified salts-treated corn
E: Salt solution treated corn

FIGURE 12. Color of Corn Samples Harvested in 1987 and Stored Eight Months (Wang, 1989)

TABLE 27. Effects of Propionate Treatments on Color of Corn Harvested with 26.8% Moisture and Stored for 12 Months

| Treatments | Application Level % | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Propionic Acid | 1.0 | $36.8^{a}$ | $9.4^{ab}$ | $14.7^{ab}$ |
| | 0.5 | $38.8^{a}$ | $9.3^{ab}$ | $15.9^{a}$ |
| Semi-Acidified Salt | 1.0 | $34.7^{a}$ | $10.2^{a}$ | $14.8^{ab}$ |
| | 0.5 | $36.8^{a}$ | $8.8^{b}$ | $13.6^{b}$ |
| Acidified Salt | 1.0 | $35.5^{a}$ | $10.0^{a}$ | $15.3^{ab}$ |
| | 0.5 | $35.6^{a}$ | $9.1^{ab}$ | $14.2^{ab}$ |
| Simul. of Ferm. Broth | 1.0 | $35.9^{a}$ | $10.2^{a}$ | $14.6^{ab}$ |
| | 0.5 | $36.3^{a}$ | $9.6^{ab}$ | $14.7^{ab}$ |
| Fermentation Broth | 1.0 | $35.5^{a}$ | $9.6^{ab}$ | $14.1^{ab}$ |
| | 0.5 | $36.9^{a}$ | $9.5^{ab}$ | $14.0^{ab}$ |

$^{ab}$Means with common superscripts in the same column are not significantly different at the 5% level.

lower total counts than those of semi-acidified salts-treated corn. However, these differences between treatments can be considered as biologically not important. Whereas the treatment effects on corn with initial moisture content of 26.8% were highly significant ($P<0.01$) and total count on propionic-acid treated corn at 1.0% application level was

TABLE 28. Effects of Propionate Treatments on Color of Corn Harvested in 1988 With 29.6% Moisture and stored for 12 months

| Treatments | Application Level % | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Propionic Acid | 1.0 | $31.4^{a}$ | $11.1^{bc}$ | $15.1^{ab}$ |
| | 0.5 | $32.1^{a}$ | $9.8^{c}$ | $14.2^{b}$ |
| Semi Acidified Salt | 1.0 | $25.3^{bc}$ | $13.3^{ab}$ | $17.8^{a}$ |
| | 0.5 | $22.2^{c}$ | $13.5^{a}$ | $17.6^{a}$ |
| Acidified Salt | 1.0 | $32.5^{a}$ | $10.3^{c}$ | $14.0^{b}$ |
| | 0.5 | $28.3^{ab}$ | $11.1^{bc}$ | $15.7^{ab}$ |
| Simul. of Ferm. Broth | 1.0 | $26.0^{bc}$ | $13.1^{ab}$ | $17.7^{a}$ |
| | 0.5 | $25.6^{bc}$ | $12.1^{abc}$ | $16.4^{ab}$ |

$^{abc}$Means with common superscripts in the same column are not significantly different at the 5% level.

lower than on semi-acidified salts-treated corn at 1.0% application level. The high count found on 1.0% semi-acidified salts-treated corn is more likely because of a contamination during the determination procedure.


F: Air-dried corn
G: Propionic acid treated corn
H: Semi-acidified salts-treated corn
I: Acidified salts-treated corn
J: Simulated fermentation broth treated corn
K: Fermentation broth treated corn

FIGURE 13. Color of Corn Samples Harvested in 1988 with 26.8% Moisture and Stored 12 months


L: Air-dried corn
M: Propionic acid treated corn
N: Semi-acidified salts-treated corn
O: Acidified salts-treated corn
P: Simulated fermentation broth treated corn

FIGURE 14. Color of Corn Samples Harvested in 1988 With 29.8% Moisture and Stored 12 Months

TABLE 29. Logarithmic Transformations of Total Count and Final Moisture of Corn After Storage[1]

| Treatments | 1987 Samples[2] Count (Log) | 1987 Samples[2] Moisture (%) | 1988 Samples[3] Count (Log) | 1988 Samples[3] Moisture (%) | 1988 Samples[4] Count (Log) | 1988 Samples[4] Moisture (%) |
|---|---|---|---|---|---|---|
| Propionic Acid | | | | | | |
| (1.0%) | 2.70[a] | 11.38 | 3.20[b] | 12.37 | 4.18[b] | 20.96 |
| (0.5%) | 2.80[a] | 12.27 | 5.66[a] | 13.45 | 4.24[b] | 19.48 |
| Semi-Acidified Salts | | | | | | |
| (1.0%) | 3.91[a] | 12.04 | 6.62[a] | 12.69 | 4.59[a] | 20.28 |
| (0.5%) | - | - | 3.44[b] | 12.02 | 4.36[ab] | 19.43 |
| Air-Dried Corn | | | | | | |
| | 3.51[a] | 13.74 | - | - | - | - |

[1]Means with common superscripts in the same column are not significantly different at the 5% level.

[2]Corn was harvested with 26.8% moisture content and stored for two years.

[3]Corn was harvested with 26.8% moisture content and stored for one year.

[4]Corn was harvested with 29.6% moisture content and stored for one year.

## 4.6. Corn Grade Evaluation

The samples from each treatment stored between one and two years were graded as U.S. sample grade due to commercially objectionable foreign odor and high total damage. The dark color of treated corn was evaluated as high total damage. Whereas air-dried corn from 1987 samples was graded as U.S. No. 1 grade.

# 5. CONCLUSIONS

The following conclusions can be drawn from this study

1) All propionate treatments, except the regular salt solution, acted as a fungicide and maintained corn in a mold-free condition for more than a year. In addition, prevented the growth of A. flavus inoculated to the corn.

2) Both the simulated fermentation broth and propionic acid fermentation broth were as effective as pure propionic acid on high moisture corn preservation

3) Color changes occurred during storage. Propionic acid maintained corn color better than other treatments and followed by acidified salt and semi-acidified salt treatments.

4) All treated samples were graded as U.S. sample grade at the end of the long term storage period due to commercially objectionable odor and high total damage.

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# 8. APPENDIX

TABLE 30. The Percentage of Kernels Infected With Fungi in Corn Harvested at 26.8% Moisture and Treated with Sodium Propionate Solution (pH 9.6)

| Weeks Stored | Plated Moldy Kernels (%) | Kernels Infected with (%) | | | |
|---|---|---|---|---|---|
| | | Aspergillus | Penicillium | Mucor and Fusarium | Yeast |
| | | 0.5% Application Level | | | |
| 0 | 60 | 14 | 4 | 50 | 0 |
| 0 | 46 | 8 | 4 | 34 | 0 |
| 0 | 54 | 12 | 8 | 32 | 0 |
| 2 | 8 | 2 | 0 | 8 | 0 |
| 2 | 6 | 0 | 0 | 6 | 0 |
| 2 | 16 | 0 | 2 | 12 | 0 |
| 4 | 80 | 0 | 8 | 80 | 20 |
| 4 | 12 | 0 | 0 | 12 | 38 |
| 4 | 74 | 4 | 10 | 74 | 78 |
| 6 | 100 | 4 | 0 | 100 | 6 |
| 6 | 100 | 6 | 0 | 100 | 20 |
| 6 | 100 | 2 | 0 | 100 | 14 |
| 8 | 100 | 52 | 0 | 100 | 0 |
| 8 | 100 | 56 | 0 | 100 | 0 |
| 8 | 100 | 34 | 0 | 100 | 0 |
| | | 1.0% Application Level | | | |
| 0 | 58 | 8 | 10 | 38 | 0 |
| 0 | 42 | 0 | 6 | 36 | 0 |
| 0 | 30 | 2 | 12 | 22 | 0 |
| 2 | 4 | 0 | 4 | 4 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 60 | 100 | 100 | 0 | 0 | 0 |
| 60 | 100 | 100 | 0 | 0 | 0 |
| 60 | 100 | 100 | 0 | 0 | 0 |

TABLE 31. The Percentage of Kernels Infected with Fungi in Corn Harvested at 23.0% Moisture and Treated with 0.5% Sodium Propionate Salt Solution (pH 9.6)

| Weeks Stored | Plated Moldy Kernels (%) | Kernels Infected with (%) | | | |
|---|---|---|---|---|---|
| | | Aspergillus | Penicillium | Fusarium | Yeast |
| 0 | 52 | 0 | 4 | 44 | 0 |
| 0 | 38 | 0 | 4 | 36 | 0 |
| 0 | 54 | 2 | 0 | 52 | 0 |
| 2 | 4 | 0 | 2 | 2 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 2 | 4 | 0 | 2 | 4 | 0 |
| 4 | 4 | 0 | 4 | 4 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 4 | 2 | 0 | 0 | 2 | 0 |
| 6 | 2 | 0 | 0 | 2 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 |
| 8 | 4 | 0 | 0 | 4 | 28 |
| 8 | 2 | 0 | 0 | 2 | 2 |
| 8 | 2 | 0 | 0 | 2 | 2 |
| 10 | 100 | 46 | 18 | 100 | 0 |
| 10 | 2 | 0 | 0 | 2 | 4 |
| 10 | 0 | 0 | 0 | 0 | 2 |
| 12 | 100 | 66 | 12 | 100 | 0 |
| 12 | 100 | 30 | 18 | 100 | 0 |
| 12 | 100 | 42 | 17 | 100 | 0 |

TABLE 32. The Percentage of Kernels Infected with Fungi in Corn Harvested at 23.0% Moisture and Treated with 1.0% Sodium Propionate Solution (pH 9.6)

| Weeks Stored | Plated Moldy Kernels (%) | Kernels Infected With (%) | | |
|---|---|---|---|---|
| | | Aspergillus | Penicillium | Fusarium |
| 0 | 40 | 0 | 2 | 38 |
| 0 | 50 | 6 | 2 | 48 |
| 0 | 42 | 0 | 2 | 42 |
| 2 | 4 | 0 | 2 | 2 |
| 2 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |

TABLE 33. The Percentage of Kernels Infected with Fungi in Corn Harvested at 17.6% Moisture and Treated with 0.5% Sodium Propionate Solution (pH 9.6)

| Weeks Stored | Plated Moldy Kernels (%) | Kernels Infected With (%) | | |
|---|---|---|---|---|
| | | Aspergillus | Penicillium | Fusarium |
| 0 | 50 | 0 | 0 | 50 |
| 0 | 56 | 2 | 0 | 56 |
| 0 | 54 | 0 | 0 | 54 |
| 2 | 38 | 2 | 2 | 38 |
| 2 | 40 | 6 | 4 | 40 |
| 2 | 36 | 6 | 4 | 34 |
| 4 | 10 | 2 | 2 | 10 |
| 4 | 16 | 4 | 4 | 16 |
| 4 | 18 | 4 | 2 | 18 |
| 6 | 4 | 2 | 0 | 2 |
| 6 | 16 | 14 | 0 | 16 |
| 6 | 6 | 6 | 0 | 6 |
| 8 | 10 | 8 | 0 | 4 |
| 8 | 8 | 4 | 0 | 4 |
| 8 | 4 | 0 | 0 | 2 |
| 10 | 2 | 2 | 0 | 0 |
| 10 | 4 | 4 | 2 | 0 |
| 10 | 8 | 8 | 2 | 2 |
| 12 | 8 | 8 | 0 | 2 |
| 12 | 8 | 8 | 2 | 0 |
| 12 | 4 | 4 | 2 | 2 |
| 14 | 8 | 8 | 2 | 2 |
| 14 | 12 | 10 | 2 | 2 |
| 14 | 8 | 4 | 2 | 2 |
| 16 | 14 | 14 | 0 | 0 |
| 16 | 18 | 16 | 2 | 2 |
| 16 | 12 | 12 | 2 | 2 |
| 18 | 24 | 22 | 4 | 0 |
| 18 | 28 | 26 | 2 | 0 |
| 18 | 30 | 28 | 2 | 0 |

TABLE 34. The Percentage of Kernels Infected with Fungi in Corn Harvested at 17.6% Moisture and Treated with 1.0% Sodium Propionate Solution (pH 9.6)

| Weeks Stored | Plated Moldy Kernels (%) | Kernels Infected With (%) | | |
|---|---|---|---|---|
| | | Aspergillus | Penicillium | Fusarium |
| 0 | 46 | 2 | 0 | 46 |
| 0 | 44 | 0 | 0 | 44 |
| 0 | 36 | 0 | 0 | 36 |
| 2 | 8 | 2 | 0 | 8 |
| 2 | 12 | 2 | 0 | 8 |
| 2 | 14 | 2 | 0 | 12 |
| 4 | 0 | 0 | 0 | 0 |
| 4 | 4 | 0 | 0 | 4 |
| 4 | 4 | 0 | 0 | 4 |
| 6 | 4 | 2 | 2 | 2 |
| 6 | 0 | 0 | 0 | 0 |
| 6 | 4 | 2 | 2 | 4 |
| 8 | 2 | 2 | 0 | 0 |
| 8 | 2 | 2 | 0 | 0 |
| 8 | 4 | 4 | 0 | 2 |
| 10 | 2 | 2 | 0 | 0 |
| 10 | 2 | 2 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |
| 36 | 0 | 0 | 0 | 0 |

TABLE 35. Hunter Colorimeter Readings on Corn Harvested in 1987 with 26.8% Moisture and Stored for 24 Months

| Treatment | Replications | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Propionic Acid | 1.0% Application Level | | | |
| | 1 | 29.13 | 13.85 | 18.15 |
| | 1 | 30.68 | 14.61 | 19.20 |
| | 1 | 28.33 | 13.76 | 17.93 |
| | 2 | 36.35 | 10.31 | 14.63 |
| | 2 | 31.54 | 9.13 | 11.72 |
| | 2 | 32.18 | 8.78 | 11.75 |
| | 0.5% Application Level | | | |
| | 1 | 30.51 | 12.44 | 16.14 |
| | 1 | 27.80 | 13.89 | 18.13 |
| | 1 | 30.71 | 13.30 | 18.00 |
| | 2 | 36.80 | 10.06 | 14.74 |
| | 2 | 37.48 | 10.42 | 14.18 |
| | 2 | 37.83 | 9.96 | 15.25 |
| Semi-Acidified Salts | 1.0 Application Level | | | |
| | 1 | 35.02 | 9.26 | 11.78 |
| | 1 | 34.96 | 8.89 | 10.73 |
| | 1 | 33.64 | 9.54 | 12.27 |
| | 2 | 33.43 | 9.31 | 10.89 |
| | 2 | 38.80 | 10.16 | 13.23 |
| | 2 | 35.40 | 9.84 | 11.45 |
| | 3 | 34.09 | 10.13 | 11.60 |
| | 3 | 37.04 | 8.66 | 11.35 |
| | 3 | 35.53 | 9.36 | 10.72 |

TABLE 36. Hunter Colorimeter Readings on Corn Harvested in 1987 with 26.8% Moisture and Stored for 24 Months

| Treatment | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Acidified Salts | | 1.0% Application Level | | |
| | 1 | 34.48 | 9.76 | 13.29 |
| | 1 | 35.59 | 9.51 | 12.56 |
| | 1 | 31.41 | 10.12 | 11.94 |
| | 2 | 33.47 | 13.53 | 18.78 |
| | 2 | 27.82 | 13.93 | 18.96 |
| | 2 | 33.48 | 12.26 | 18.31 |
| | 3 | 33.45 | 10.89 | 13.70 |
| | 3 | 35.80 | 12.75 | 16.90 |
| | 3 | 34.20 | 10.11 | 15.60 |
| | | 0.5% Application Level | | |
| | 1 | 38.16 | 9.94 | 15.51 |
| | 1 | 39.19 | 10.69 | 15.95 |
| | 1 | 38.78 | 8.34 | 14.05 |
| | 2 | 30.75 | 13.61 | 18.95 |
| | 2 | 31.83 | 14.90 | 19.31 |
| | 2 | 33.39 | 14.25 | 19.79 |
| | 3 | 39.60 | 8.60 | 15.20 |
| | 3 | 40.50 | 10.18 | 16.80 |
| | 3 | 39.69 | 9.80 | 16.54 |

TABLE 37. Hunter Colorimeter Readings on Air-dried Corn Harvested in 1987 and Stored for 24 Months[a]

| Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|
| 1 | 53.71 | 14.50 | 21.83 |
| 1 | 51.80 | 15.50 | 23.60 |
| 1 | 53.31 | 14.13 | 22.85 |
| 2 | 41.87 | 18.20 | 23.30 |
| 2 | 42.30 | 18.63 | 25.61 |
| 2 | 42.22 | 18.58 | 25.49 |
| 3 | 48.79 | 17.70 | 22.70 |
| 3 | 48.60 | 15.35 | 21.61 |
| 3 | 49.22 | 16.66 | 22.29 |

[a]The corn harvested with 25.0% moisture and air-dried to 12.0% Moisture.

TABLE 38. Hunter Colorimeter Readings on Corn Harvested in 1988 with 26.8% Moisture and Stored for 12 Months

| Treatment | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Propionic Acid | | 1.0% Application Level | | |
| | 1 | 34.44 | 10.37 | 14.57 |
| | 1 | 38.08 | 10.35 | 16.20 |
| | 1 | 41.32 | 8.41 | 15.83 |
| | 2 | 34.86 | 9.94 | 14.15 |
| | 2 | 31.51 | 9.27 | 13.15 |
| | 2 | 37.05 | 8.10 | 14.04 |
| | 3 | 38.39 | 8.11 | 13.99 |
| | 3 | 37.61 | 9.79 | 15.09 |
| | 3 | 37.57 | 10.33 | 15.52 |
| | | 0.5% Application Level | | |
| | 1 | 37.25 | 9.31 | 15.03 |
| | 1 | 35.71 | 8.93 | 13.00 |
| | 1 | 36.44 | 8.33 | 13.98 |
| | 2 | 39.79 | 10.54 | 17.81 |
| | 2 | 41.08 | 9.81 | 18.48 |
| | 2 | 42.78 | 8.67 | 17.04 |
| Semi-Acidified Salts | | 1.0% Application Level | | |
| | 1 | 33.26 | 9.92 | 14.23 |
| | 1 | 35.04 | 10.58 | 15.12 |
| | 1 | 33.77 | 10.29 | 14.17 |
| | 2 | 38.75 | 8.89 | 16.03 |
| | 2 | 34.44 | 10.29 | 14.91 |
| | 2 | 33.95 | 10.17 | 14.71 |
| | 3 | 34.06 | 9.21 | 13.97 |
| | 3 | 33.08 | 10.98 | 14.55 |
| | 3 | 36.02 | 11.19 | 15.70 |
| | | 0.5% Application Level | | |
| | 1 | 36.87 | 8.87 | 13.10 |
| | 1 | 38.42 | 7.56 | 12.50 |
| | 1 | 39.15 | 8.04 | 13.13 |
| | 2 | 40.09 | 8.98 | 15.11 |
| | 2 | 33.69 | 9.12 | 12.97 |
| | 2 | 37.97 | 9.38 | 15.02 |
| | 3 | 34.23 | 8.45 | 12.42 |
| | 3 | 35.31 | 9.68 | 14.25 |
| | 3 | 35.26 | 9.10 | 13.81 |

TABLE 39. Hunter Colorimeter Readings on Corn Harvested in 1988 with 26.8% Moisture and Stored for 12 Months

| Treatment | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Acidified Salts | | 1.0% Application Level | | |
| | 1 | 33.32 | 10.48 | 14.61 |
| | 1 | 35.37 | 9.09 | 14.35 |
| | 1 | 32.58 | 9.31 | 12.92 |
| | 2 | 33.27 | 10.60 | 14.79 |
| | 2 | 33.20 | 11.12 | 15.14 |
| | 2 | 35.45 | 8.99 | 15.15 |
| | 3 | 40.47 | 10.36 | 17.43 |
| | 3 | 36.10 | 9.85 | 15.83 |
| | 3 | 40.09 | 9.99 | 17.19 |
| | | 0.5% Application Level | | |
| | 1 | 33.27 | 8.69 | 12.70 |
| | 1 | 37.82 | 9.00 | 14.97 |
| | 1 | 34.08 | 8.78 | 13.04 |
| | 2 | 35.02 | 11.06 | 16.03 |
| | 2 | 32.61 | 9.86 | 13.62 |
| | 2 | 39.22 | 9.34 | 15.44 |
| | 3 | 36.49 | 7.81 | 14.34 |
| | 3 | 36.69 | 8.51 | 14.41 |
| | 3 | 34.74 | 8.68 | 13.62 |
| Simulated Fermentation Broth | | 1.0% Application Level | | |
| | 1 | 38.64 | 10.45 | 15.70 |
| | 1 | 39.60 | 10.24 | 15.67 |
| | 1 | 37.03 | 9.73 | 14.81 |
| | 2 | 31.14 | 9.71 | 12.63 |
| | 2 | 34.52 | 10.41 | 14.40 |
| | 2 | 34.46 | 10.94 | 14.66 |
| | | 0.5% Application Level | | |
| | 1 | 36.39 | 9.74 | 15.04 |
| | 1 | 34.87 | 9.08 | 13.36 |
| | 1 | 37.17 | 10.93 | 15.68 |
| | 2 | 35.91 | 9.55 | 14.60 |
| | 2 | 33.87 | 7.90 | 12.86 |
| | 2 | 35.50 | 8.73 | 14.62 |
| | 3 | 37.67 | 9.75 | 15.29 |
| | 3 | 40.78 | 10.50 | 17.16 |
| | 3 | 34.95 | 10.02 | 14.10 |

TABLE 40. Hunter Colorimeter Readings on Corn Harvested in 1988 with 26.8% Moisture and Stored for 12 Months

| Treatment | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Fermentation Broth | | 1.0% Application Level | | |
| | 1 | 36.24 | 9.80 | 14.59 |
| | 1 | 35.71 | 9.02 | 14.12 |
| | 1 | 34.28 | 9.22 | 13.11 |
| | 2 | 35.24 | 9.22 | 14.50 |
| | 2 | 36.33 | 10.15 | 15.00 |
| | 2 | 35.00 | 9.90 | 13.00 |
| | | 0.5% Application Level | | |
| | 1 | 34.07 | 9.22 | 12.99 |
| | 1 | 39.31 | 9.34 | 15.66 |
| | 1 | 37.26 | 9.00 | 13.54 |
| | 2 | 38.42 | 9.15 | 13.40 |
| | 2 | 35.22 | 10.24 | 14.70 |
| | 2 | 37.24 | 10.18 | 13.50 |

TABLE 41. Hunter Colorimeter Readings on Corn Harvested in 1988 with 29.6% and Stored for 12 Months

| Treatments | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Propionic Acid | | 1.0% Application Level | | |
| | 1 | 32.35 | 12.85 | 18.97 |
| | 1 | 27.27 | 12.46 | 17.52 |
| | 1 | 28.56 | 13.18 | 17.71 |
| | 2 | 31.95 | 9.70 | 13.03 |
| | 2 | 33.32 | 9.33 | 12.75 |
| | 2 | 35.22 | 9.28 | 13.02 |
| | 3 | 31.35 | 11.47 | 15.60 |
| | 3 | 32.95 | 9.85 | 12.89 |
| | 3 | 29.96 | 11.85 | 14.50 |
| | | 0.5% Application Level | | |
| | 1 | 35.62 | 8.23 | 12.50 |
| | 1 | 34.97 | 8.48 | 12.39 |
| | 1 | 37.05 | 9.09 | 13.81 |
| | 2 | 30.67 | 9.72 | 12.42 |
| | 2 | 35.81 | 8.56 | 13.50 |
| | 2 | 35.03 | 8.87 | 13.81 |
| | 3 | 26.92 | 12.36 | 17.57 |
| | 3 | 24.75 | 11.54 | 15.40 |
| | 3 | 27.97 | 11.52 | 16.43 |
| Semi-Acidified Salts | | 1.0% Application Level | | |
| | 1 | 29.58 | 13.41 | 19.23 |
| | 1 | 24.20 | 13.07 | 17.20 |
| | 1 | 22.92 | 13.11 | 17.86 |
| | 2 | 24.63 | 13.31 | 17.42 |
| | 2 | 27.10 | 12.03 | 16.31 |
| | 2 | 26.33 | 13.18 | 16.64 |
| | 3 | 24.21 | 12.55 | 17.35 |
| | 3 | 26.99 | 14.36 | 19.36 |
| | 3 | 21.95 | 14.23 | 18.75 |
| | | 0.5% Application Level | | |
| | 1 | 22.13 | 13.85 | 17.50 |
| | 1 | 23.72 | 13.10 | 17.30 |
| | 1 | 27.15 | 14.46 | 19.15 |
| | 2 | 19.21 | 13.92 | 17.17 |
| | 2 | 20.13 | 12.40 | 15.87 |
| | 2 | 23.77 | 11.36 | 16.57 |
| | 3 | 20.45 | 13.86 | 18.16 |
| | 3 | 17.65 | 16.63 | 19.87 |
| | 3 | 25.38 | 12.27 | 17.10 |

TABLE 42. Hunter Colorimeter Readings on Corn Harvested in 1988 with 29.6% Moisture and Stored for 12 Months

| Treatment | Replicates | 'L' Value | 'a' Value | 'b' Value |
|---|---|---|---|---|
| Acidified Salts | | 1.0% Application Level | | |
| | 1 | 32.06 | 11.15 | 14.92 |
| | 1 | 29.41 | 10.47 | 12.32 |
| | 1 | 32.16 | 10.14 | 13.35 |
| | 2 | 32.41 | 8.92 | 12.79 |
| | 2 | 35.81 | 10.63 | 15.36 |
| | 2 | 34.17 | 10.32 | 15.04 |
| | 3 | 27.22 | 9.66 | 11.89 |
| | 3 | 34.91 | 10.56 | 14.75 |
| | 3 | 34.62 | 10.94 | 14.89 |
| | | 0.5% Application Level | | |
| | 1 | 25.20 | 13.56 | 18.62 |
| | 1 | 25.74 | 10.99 | 15.84 |
| | 1 | 29.26 | 11.51 | 17.21 |
| | 2 | 32.04 | 8.23 | 11.63 |
| | 2 | 33.19 | 8.60 | 12.64 |
| | 2 | 31.75 | 8.42 | 11.91 |
| | 3 | 25.87 | 13.05 | 18.27 |
| | 3 | 23.78 | 12.13 | 16.62 |
| | 3 | 27.76 | 12.93 | 18.54 |
| Simulated Fermentation Broth | | 1.0% Application Level | | |
| | 1 | 28.75 | 12.41 | 17.71 |
| | 1 | 22.07 | 13.43 | 17.69 |
| | 1 | 27.97 | 13.30 | 18.75 |
| | 2 | 22.97 | 12.38 | 17.16 |
| | 2 | 27.78 | 13.58 | 18.63 |
| | 2 | 27.94 | 13.99 | 18.83 |
| | 3 | 23.88 | 12.61 | 16.20 |
| | 3 | 27.32 | 13.88 | 18.46 |
| | 3 | 25.00 | 12.39 | 16.15 |
| | | 0.5% Application Level | | |
| | 1 | 28.53 | 11.36 | 16.53 |
| | 1 | 26.71 | 11.27 | 15.97 |
| | 1 | 26.10 | 12.39 | 16.87 |
| | 2 | 22.95 | 12.04 | 16.90 |
| | 2 | 23.06 | 13.52 | 17.28 |
| | 2 | 25.62 | 10.53 | 15.27 |
| | 3 | 27.44 | 12.54 | 16.95 |
| | 3 | 25.32 | 11.95 | 16.30 |
| | 3 | 24.43 | 12.90 | 15.90 |

TABLE 43. Total Aerobic Counts Present in All Purpose Tween Agar (CFU/g)

| Treatments | 1987 Samples[a] | 1988 Samples[b] | 1988 Samples[c] |
|---|---|---|---|
| Propionic Acid (1.0%) | 1000 est | $18x10^3$ | 1400 est |
| | 250 est | $13x10^3$ | 1800 est |
| (0.5%) | 400 est | $20x10^3$ | $13x10^5$ |
| | 1000 est | $15x10^3$ | $16x10^4$ |
| Semi-Acidified Salts (1.0%) | $96x10^3$ | $49x10^3$ | $11x10^5$ |
| | 700 est | $31x10^3$ | $16x10^6$ |
| (0.5%) | - | $23x10^3$ | $25x10^2$ |
| | - | $23x10^3$ | $31x10^2$ |
| Air-Dried Corn | 1350 est | - | - |
| | $79x10^2$ | - | - |

[a]Corn was harvested with 26.8% moisture and stored for two years.

[b]Corn was harvested with 29.6% moisture and stored for one year.

[c]Corn was harvested with 26.8% moisture and stored for one year.