CHEMICAL PRESERVATION OF HIGH MOISTURE CORN AND EFFECTS OF PELLETING AND CHEMICALS ON AFLATOXIN

by

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INTRODUCTION

The role of fungi in causing the deterioration of cereal grains is well documented. Storage fungi cause loss of germination, dark germs, bin burning, mustiness and heating. Some of the storage fungi produce toxins. A major factor in mold invasion of grain is moisture content (1).

With mechanized harvesting practices, preservation of high moisture grain adds a new dimension to the problem of grain storage. The traditional method of drying high moisture grain to safe moisture levels has disadvantages because of cost, availability of equipment and the necessity of investment in equipment to be used for only a short period of time. A recent development for handling high moisture grain involves use of organic acids, chiefly propionic and acetic acids (2). Poor results due to negligence or other causes has resulted in moldy grain. In such cases, the major consideration in recommending disposal of the grain is the possible presence of toxins such as aflatoxins. Attempts to eliminate aflatoxin by ensiling the moldy grain were not successful (3). Partial success was reported with milling (4). Solvent extraction, exposure to moist heat, treatment with gaseous reagents such as ammonia and alkali treatment are some of the other methods suggested (5).

The purpose of the present investigation was to study the efficiency of sorbic acid and Grain Treet[R]*. These were studied in comparison to propionic acid. Acetic acid and calcium propionate were also studied for comparisons. Also studied was the effectiveness of pelleting and alkali treatment, separately or together for reducing the concentration of aflatoxin in feed made from moldy corn.

REVIEW OF LITERATURE

Grain storage with reference to mold control:

Studies concerned with grain preservation include those of Milner et. al. on the influence of certain mold inhibitors on the respiration of moist wheat (moisture content, 20%). Chemicals listed by them in the order of their decreasing value were 8-hydroxyquinoline-sulfate, Thiourea, p-amino-benzoic acid, sulfanilamide, benzene sulfonamide, 2-amino-thiazole, chloramine B, and calcium propionate (6). Most of these chemicals with the exception of Ca-propionate, are either toxic to animals or too expensive for grain treatment. In another study Matz and Milner (7) reported that carbon tetra-chloride and propylene glycol were the most effective among a number of chemicals tested on damp wheat. However, they reported that none of the chemicals counteracted entirely the

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*A commercial product of Kemin Ind., Inc., Des Moines, Iowa. (Active ingredients: 20% propionic acid; inert ingredients: 80%:acetic acid, propyl p-hydroxybenzoate, benzoic acid and sorbic acid.)

damaging effects of high moisture grain storage even though the mold growth was sometimes inhibited. They concluded that chemical treatments might be useful for short-term emergency storage until the grain could be dried to safe moisture levels.

The fungitoxicity of alcohols and amines, as a function of their chain length was explored in connection with preservation of wood, leading to a postulation that fungitoxic material have two fundamental modes of action, viz., one inside the cell membrane and one outside of the membrane. Internal fungitoxicants are held to be limited to a chain length of about 12-carbons regardless of water solubility. External fungitoxicants possibly act by denaturing cellulolytic enzymes and, therefore, can be either water-soluble or water-insoluble chemicals (8). This article is of interest since the surface of grain and wood share some common features.

Richardson and Halick (9) have assayed various compounds for their heat-inhibiting activity with corn meal of moisture content ranging from 14-15%. They concluded that propionic acid and propionic anhydride were effective at a level of 0.1%, calcium propionate at a level of 0.3%, whereas Na-propionate, propionamide and propionalide were not effective. They also checked fatty acids for their mold inhibiting activity and concluded that their heat inhibiting activity decreases as the number of carbons in the chain increases. Srinivasan and Majumder have reported des-

truction of molds in Kafir corn at 20% moisture following fumigation with methyl bromide and ehtylene bromide and hermetic storage for 60 days at 25-29°C. They have also found encouraging results with chloropicrin and ethylene oxide (10). The use of fumigants calls for expensive storage structures and may create the problem of residues in the grain.

Organic fungicides for plant protection have been used for some time. They include organo-metal compounds, unnatural forms of amino acids such as D-forms of serine and threonine and the L-form of threo-B-phenyl-serine, Thiourea, and dithiocarbamic acid derivatives (11). Some of these compounds are not desirable as food ingredients and hence can be eliminated as grain preservatives.

Sodium propionate is reported to reduce infection by storage fungi on rough rice dried by infrared radiation to 12.7 to 13.0%. The protection was said to last for 6 months when stored at 75% R.H. and for 4 months when stored at 85% R.H. (12). Deyoe and Quadri have reported that Mold Curb* applied at a rate of 0.1% resulted in a significant decrease of mold population of pelleted feed (13).

Sorbic acid was reported as harmless to dogs and rats at a level of 5% and can be utilized as a source of calories. When compared to sodium benzoate, it was found to be far less toxic (14). Studies connected with the metabolism of sorbic acid

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*A commercial product of Kemin Ind., Inc., Des Moines, Iowa. (Active ingredient: 20% propionic acid.)

by fungi have revealed that sorbic acid was related to caproic acid in furnishing carbon for mold growth in the same manner as crotonic acid was related to butyric acid. Evidence was presented to show that sorbic acid by being an α,β-unsaturated acid inhibits the dehydrogenase system in the mold. However, this inhibition was reversible in the presence of high mold population and comparatively lower sorbic acid concentration. Hence sorbic acid cannot protect foods prepared unhygienically. Mold inhibition with sorbic acid was a function of dosage (15). Sorbic acid in a concentration of 0.05-0.1% was found to strongly delay the onset of mold and yeast growth in smoked fish, whereas chlortetracycline and tetracycline were not at all effective (16). Sorbic acid at a concentration of 0.075% was found to inhibit for a period of 14 days, strains of Aspergillus, Pencillium, Alternaria, and Mucor species isolated from tomato and strawberry fruits. Also comparisons at pH levels of 3.3 and 4.0 proved that sorbic acid was more effective at the lower pH level (17). The results obtained with artificial media, however, may have little correlation to what happens in grains. In a study conducted with cakes, sorbic acid was found to be approximately four times as effective as calcium or sodium propionate and sodium benzoate. When the above were used at concentrations of 0.2%, sorbic acid extended the mold-free life of the cakes to four times that of propionate (18). In another study, sorbic acid added at a level of 0.3% to corn meal containing 16.3% moisture, prevented heating for

42 days whereas at a level of 0.1%, and 0.2%, it delayed heating 3 to 17 days (0.1%) and 33 days (at 0.2%), (19). In a more recent study concerned directly with the preservation of high moisture grain, acetic acid, propionic acid, and sorbic acid at concentrations of 0.7%, 0.4%, and 0.08% respectively, inhibited the development of mold in broth cultures. When applied to grain sorghum and corn adjusted to 18% and 24% moisture respectively, sorbic acid had no beneficial effect even at 1.0% concentration, whereas corn treated with 1.0% acetic acid or 0.6% propionic acid remained mold free during 2 months of storage. The report also indicated an absence of a synergistic effect between acetic and propionic acids. A number of other organic chemicals tested did not produce beneficial effects (19).

Aflatoxin elimination studies:

The literature covering the various aspects of aflatoxins is extensive (20). Dollear has discussed methods and criteria for detoxification of aflatoxins in foods and feeds. The methods he has discussed included alkali refining of vegetable oils, physical separation of damaged kernels, heat treatment, solvent extraction, chemical inactivation and microbiological inactivation (21). A temperature of 300°C.or above is required to effect thermal degradation of isolated aflatoxins (22). But given relatively higher moisture content, and, if possible, pressure also, aflatoxins present in feed ingredients could be

partially broken down (23, 24, 25). A host of chemicals, for example, ammonia, methylamine, sodium hydroxide, hydrogen peroxide, ozone, etc., are found to be capable of inactivating aflatoxin given sufficient concentration (21, 27). Since reactions with alkali are reversible, results based on fluorescence needs substantiation by biological assays, or by mild acidification to determine if fluorescence reappears (23, 28).

Preliminary trials in our laboratory indicated the usefulness of sorbic acid as a fungistatic agent for the preservation of high moisture corn grain.

There are no reports relating pelleting to the breakdown of aflatoxins. It is not possible to predict what effects pelleting might have on the destruction of aflatoxins since some of the factors involved in pelleting such as pelleting temperature, pelleting pressure, feed time in die vary over a wide range (26). However, comparison of data by others (25, 26) suggest a possible beneficial effect of pelleting.

MATERIALS AND METHODS

Yellow corn with an average initial moisture content of 15% was bought from a local grain elevator. Water was added to bring the moisture content to 25% by conditioning in a horizontal tumbling mixer for 6 hrs. The grain was stored at room temperature (70°F.) for 18 hrs. prior to treatment. Each treatment was carried out in duplicate. Two kg. of reconstituted high moisture corn was spread on a plastic sheet to form a 1 cm. thick layer. The test chemical was mixed with a suitable carrier (ethanol in the case of sorbic acid, and water in the case of others) and sprayed onto the grain using an atomizer. Total spray volume was 5% by weight of the grain. Samples were then taken for pH measurement and for storage in a humidity chamber. The remaining grain was then transferred to metal cans (2 kg. capacity), lined with plastic bags. The bags were sealed after placing a beaker of water over the grain column. A thermocouple with a length approximately equal to the depth of the grain column was inserted through a small hole in the plastic sheet with the sensor in the center of the grain column in each can. The cans were stored for the first 6 days at approximately 72°F and then transferred to the 2nd flour of the feedmill, where the temperatures were near outside temperatures. The experiment was conducted during the summer months of 1971. Temperatures of the atmosphere and the

grain were recorded once daily for a period of 30 days for all samples except those where the grain mass became heavily molded in the first few days.

The portion of each treatment (approx. 100 g.) taken for the humidity chamber, was stored in glass beakers filled to the top, at 100% relative humidity in partially aerated chambers (forced aeration was not used). The onset of mold was visually observed.

At the end of the 30-day experimental period, samples were taken for estimating the percentage of grains invaded by various fungi and the germination capability of treated, non-moldy grain.

Three sets of experiments were run consecutively with the same model as above with modifications as follows: the grain meant for sorbic acid treatment with ethanol as a carrier had an extra 5% of water added to equalize the 5% water added in all other treatments for dissolving the chemical. In all samples, the moisture content at the end of the conditioning and treatment varied between 26-28%.

Propionic acid and acetic acid used in the experiment were of analytical reagent grade (Mallinckrodt Chemical). Kemin's Experimental Product, and Grain Treet^{R} were the products of Kemin Ind., Des Moines, Iowa. Temperatures of the grain were monitored using a Telethermometer (Yellowsprings Instrument Co., Inc.).

Moisture content was determined by the method of Hart et.al. (29), and pH was estimated by soaking 25 g. of the corn in 50 ml. of distilled water of known pH, for ½ hr. and then measuring the pH of the supernatant using a Leeds and Northrup model 7 pH meter. Germination was estimated by placing 100-200 whole kernels between wet towels, wrapped with aluminum foil and incubated at room temp. (approx. 25°C). Seeds sprouting on or before the 8th day were considered germinated. Fungi were detected as follows: the kernels were washed in 2% sodium hypochlorite for one minute, rinsed with sterile distilled water (30), placed on malt salt agar (4% NaCL) with Tergitol* (200 ppm), for 6 days at room temp. The mold species were identified and expressed as percentage of grains infected.

Aflatoxin for addition to feed was prepared in the laboratory by inoculation of moist sterilized rice media with Aspergillus parasiticus (NRRL 2999) as recommended by Shotwell et.al. (31). Aflatoxin was assayed by a modified method of Pons et. al (32). Visual comparisons with standards were made for quantitation.

Water-soluble acidity in feed samples was estimated by the method given in AOAC 22:053 (33). Apart from titration with sodium hydroxide, pH of the filtrate was also measured using a Leeds and Northrup pH meter.

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*Union Carbide Corp.

Fifteen pounds of toxic feed material containing approximately 5,000 p.p.b. of aflatoxin was prepared from the original rice media. This was mixed for ½ hr. with 150 lbs. of p-17 (K.S.U. chick grower ration), in a horizontal ribbon mixer. The mixture was then divided into 3 equal parts of 50 lbs. each. Each fifty lbs. was then mixed with 100 lbs. of fresh p-17 for 15 min. This procedure resulted in a total of 450 lbs. of aflatoxin contaminated feed. From this, 150 lbs. was pelleted at a conditioning temperature of 80°C. without added chemical (Batch I). A second 150 lbs. portion was treated with 2% sodium hydroxide (3 lbs. in 3 liters of water) by adding the chemical to the feed in a horizontal mixer and mixing for a total of 5 minutes. This treatment was carried out just prior to pelleting (Batch II). The third 150 lbs. portion (Batch III) was treated with 2% ammonium hydroxide in the same manner as for Batch II. Sampling was carried out at 3 points for each batch (4 samples at each point) as indicated below:

1. After the addition of toxin and chemical (except in Batch I);
2. After conditioning, but before pelleting;
3. After pelleting and cooling.

NaOH and $(NH_4)OH$ were reagent grade A.C.S.

RESULTS AND DISCUSSION

Table I gives data pertaining to the preliminary studies conducted with high moisture corn. It should be noted that potassium sorbate, a water-soluble compound was not an effective fungistat. Table II gives the pH of the chemical mixture applied to the grain and the resulting pH of the grain immediately after treatment. In accompanying graphs the heat inhibiting activity of various chemicals is illustrated. Figure 1 shows the ambient temperatures for the three sets of experiments conducted. Though the environmental temperature was monitored on a continuous basis, only the temperatures existing at the time of recording the temperatures of the grain mass were plotted. They are for the most part temperatures at 12:00 noon.

In subsequent graphs, the temperature difference between the grain and that of the environment was plotted to eliminate the variability induced by ambient temperatures for the three sets. This presentation also permits comparisons between the treatments belonging to different sets since all other conditions were identical including the grain.

Comparison of Figures 2 and 3 indicates that propionic acid was more effective than acetic acid and calcium propionate when applied at similar concentrations. Figures 4, 5 and 6 show that sorbic acid was an effective mold inhibitor for high moisture grain with effects like propionic acid. Sorbic acid

at 0.1% was as effective as 1.0% propionic acid in heat-inhibiting activity under the experimental conditions used and the time involved. It was observed that increasing the concentration of sorbic acid from 0.1-0.5% did not result in major differences in heat-inhibiting activity. Figures 7, 8 and 9 indicate Grain Treet was superior to Kermin's Experimental Product and Grain Savor in heat-inhibiting activity in high moisture grain. Increasing the level of Grain Treet from 0.6% to 1.0% indicated only a slight advantage during the 30-day observation period. Grain Treet applied at the rate of 0.6% was equal or slightly more effective than 1.0% propionic acid as a heat inhibiting agent for high moisture corn. Figures 10 and 11 indicate the superiority of Grain Treet over acetic acid and calcium propionate. These results must be considered to apply to the experimental conditions used which were near anaerobic since the tops of the plastic bags were kept closed around the thermocouple wire. Under the test conditions it was not possible to pinpoint the exact time that mold growth began in those treatments that did not preserve grain since the cans were not disturbed till the end of the study. The controls with no treatment were an exception as moldiness was apparent in the first few days. The data on mold populations in various treatments at the end of 30-days confirms the correlation between heat-inhibiting activity and fungistatic or fungicidal efficiency.

Sorbic acid (0.1-0.5%) and 1.0% propionic acid were effective in reducing mold population whereas 1.0% calcium propionate and 1.0% acetic acid were not effective (Table III). Kemin's Experimental Product and Grain Savor lacked the effectiveness of Grain Treet (Tables IV and V). These data also indicate that concentrations of a mold-inhibiting chemical below effective levels may result in the promotion of mold growth.

The results obtained with sorbic acid in this experiment are in contrast to those obtained by Simon (19) who reported little effect from sorbic acid. Simon used wheat flour as a carrier, whereas, in the present case, ethanol was employed as a carrier to effect complete solubilization of sorbic acid. The application of sorbic acid in soluble form may have enhanced its effect. In this connection, it may be mentioned that potassium sorbate, a water-soluble compound, was ineffective because sorbic acid was effective only in acid medium. Comparison of pH values in Tables I and II illustrates this point.

The data obtained on samples stored at 100% relative humidity and nearly aerobic conditions indicated differences due to the test procedure. Table VI shows the time until the onset of mold growth for the various treatments and the presence or absence of mold in their counterparts stored in the cans under an anaerobic situation. Also included in

the table are the results of germination tests for those treatments that were tested.

Tables VII, VIII and IX indicate the nature of the fungal population in the various treatments stored at 100% R.H. The most important difference observed was that sorbic acid failed to protect the grain from fungi until the concentration reached 0.5%. The lack of inhibition by sorbic acid at concentrations below 0.5% may have been due to metabolic compensation by mold in the presence of available oxygen or to metabolism of sorbic acid at lower levels.

In summary: 1) the grain used in the three sets of experiments came from the same source; 2) reconstituted grain has been reported more susceptible to mold damage, thus data obtained with such grain can be easily applied to high moisture grain obtained from the field; and 3) though ambient temperatures for the three sets vary, each treatment was tested under the same conditions.

The data on germination from Table VI indicates that treatments effective in controlling mold were not capable of retaining germination.

Grain Treet at concentrations of 0.6% or more and propionic acid at 1.0% were effective grain preservatives at moisture levels near 25%. Large scale, longer term trials are required to determine the influence of concentration on the length of storability and effects of grain preservatives

on palatability, digestion and grain storage and handling equipment.

Table X gives the data on the effect of pelleting and chemicals on aflatoxin breakdown. Under normal operating conditions of pellet mill, viz., length of conditioning, temperature of conditioning (80°C. in this case), and pressure, there is only an insignificant reduction in toxin content. The addition of 2.0% sodium hydroxide or 2.0% ammonium hydroxide did not produce beneficial effects. These results are not encouraging when compared with the data obtained by Mann et.al. (27). This may be due to a shorter period of contact between the chemical and aflatoxin (the transit time in the conditioning chamber of pellet mill is short compared to a cooking time of 2 hrs. at 100°C. in the procedure adopted by Mann et.al.) and/or due to a nonspecific reaction between sodium hydroxide or ammonium hydroxide, as the case may be, with some of the dietary component other than aflatoxin. The experiment conducted by Mann et.al. involved the use of pure cottonseed meal or peanut meal compared to the complete feed used in this case. Table XI gives the composition of diet used in this experiment.

The apparent reduction in aflatoxin content of sodium hydroxide treated feed, even before the pelleting operation, could be due to masking of fluorescence caused by sodium hydroxide, rather than a real breakdown of aflatoxin. Such a thing is known to occur (28), and the increase in the pH of sodium

hydroxide treated meal attests to such a possibility. We did not attempt to clarify this point further, because the aflatoxin content of the final product was not affected by the treatment.

The results reported here do not exclude the possibility of achieving beneficial effects, if the operating conditions of pellet mill could be made to approach the optimum conditions recommended by Mann et.al. (27), unless an interaction between the added chemical and dietary component does occur. Such severe conditions are known to reduce feed conversion efficiency and hence cannot be used as a routine procedure to safeguard feed from the possible presence of aflatoxin, but could be applied when using feed ingredients known to contain limited amounts of aflatoxin.

TABLE I. COMPARISON OF FUNGISTATICITY OF VARIOUS ORGANIC CHEMICALS IN HIGH MOISTURE CORN

(AV. MOIST. CONTENT 26%)

Treatment		pH of the Treated Grain	Visual Appearance After one week*	Remarks
No treatment	A	5.10	+++	98% of the grains
	B	5.00	+++	infected with A. flavus.
Propionic Acid 1.5%	A	3.75	---	
	B	3.70	---	
Propionic Acid 1.0%	A	3.90	---	
	B	3.85	---	
Propionic Acid 0.75%	A	4.00	---	
	B	3.95	---	
Propionic Acid 0.50%	A	4.10	(±)	
	B	4.00		
Potassium Sorbate 1.0%	A	5.65	+++	94% of the grains
	B	5.75	+++	infected with A. flavus.
Calcium Propionate 1.0% Citric Acid (1:1)	A	4.10	(±)	
	B	4.00		
Ethanol 1.5%	A	4.75	++	
	B	4.85	++	
Ethanol 1.0%	A	5.10	++	
	B	5.10	++	

* +++ Heavily invaded by mold, ++ Moderately invaded, + lightly invaded
--- Absence of mold invasion, ± Suspicious

TABLE II. AN OUTLINE OF SETUP OF VARIOUS TREATMENTS IN DIFFERENT SETS

Treatment No.	Description of the Chemical	pH of the Chemical Solution Applied	pH of the Grain after Treatment
SET I.			
1.	Control	5.60	5.35
2.	Ethanol 5.0%	5.80	5.50
3.	Sorbic acid 0.1%	4.30	4.95
4.	Sorbic acid 0.2%	4.30	4.70
5.	Sorbic acid 0.3%	4.10	4.55
6.	Sorbic acid 0.4%	3.90	4.35
7.	Sorbic acid 0.5%	3.75	4.35
8.	Propionic acid 1.0%	2.40	4.12
9.	Acetic acid 1.0%	2.20	3.90
10.	Calcium propionate 1.0%	7.70	5.62
SET II.			
1.	Kemin's Exp. Product 0.1%	2.15	4.77
2.	Kemin's Exp. Product 0.5%	1.72	4.25
3.	Grain Treet 0.1%	2.25	4.65
4.	Grain Treet 0.5%	1.67	4.30
5.	Kemin's Exp. Product 1.0%	1.40	3.97
6.	Grain Treet 1.0%	1.45	4.07
SET III.			
1.	Kemin's Exp. Product 0.6%	1.60	4.40
2.	Kemin's Exp. Product 0.7%	1.50	4.30
3.	Kemin's Exp. Product 0.8%	1.45	4.25
4.	Kemin's Exp. Product 0.9%	1.40	4.20

Contd.

TABLE II. Contd.

5.	Grain Treet 0.6%	1.57	4.40
6.	Grain Treet 0.7%	1.55	4.40
7.	Grain Treet 0.8%	1.50	4.30
8.	Grain Treet 0.9%	1.42	4.30
9.	Grain Savor (New) 0.1%	3.00	4.90
10.	Grain Savor (New) 0.2%	3.20	4.90
11.	Grain Savor (New) 0.3%	3.10	4.90
12.	Grain Savor (New) 0.4%	3.05	4.85
13.	Grain Savor (New) 0.5%	3.02	4.80
14.	Grain Savor (New) 1.0%	3.05	4.55

N.B. The values reported above were the averages of the duplicates.

TABLE III. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT AND 80°F.

% of Surface Sterilized Grains Infected*

Sample Description	Fus.	A.fl.	A.ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor.	No fungi
Control	88	56	2	4	2	0	6	30	2
Ethanol	60	70	2	4	28	0	0	0	0
Sorbic acid 0.1%	10	2	0	0	0	0	0	0	90
Sorbic acid 0.2%	0	0	0	0	0	0	0	0	100
Sorbic acid 0.3%	2	0	0	0	0	0	0	0	98
Sorbic acid 0.4%	0	0	0	0	0	0	0	0	100
Sorbic acid 0.5%	0	0	0	0	0	0	0	0	100
Propionic acid 1.0%	0	0	0	0	0	0	0	0	100
Calcium propionate 1.0%	34	96	2	0	0	0	0	0	0
Acetic acid 1.0%	86	60	0	0	0	0	0	0	2

* The data given above refers to the samples obtained from grain stored in cans under anaerobic conditions, and the values are the averages of the duplicates.

Fus. = Fusarium
A.fl.= Aspergillus flavus
A.ni.= Aspergillus niger
A.gl.= Aspergillus glaucus
A.ter.= Aspergillus terreus
Pen. = Penicillium
Rhi. = Rhizopus

TABLE IV. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT AND 80°F.[1/]

% of Surface Sterilized Grains Infected

Sample Description	*Fus.	A.fl.	A.ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor	No fungi
Kemin's Exp. Product 0.1%	38	96	0	0	0	0	30	4	0
Kemin's Exp. Product 0.5%	48	100	0	6	0	0	6	10	0
Grain Treet 0.1%	54	90	4	0	0	0	24	2	0
Grain Treet 0.5%	22	100	0	0	0	2	0	2	0
Kemin's Exp. Product 1.0%	44	100	0	0	0	0	0	2	0
Grain Treet 1.0%	0	0	0	0	0	0	0	0	100

[1/] The data given above refers to the samples obtained from grain stored in cans under anaerobic conditions. The values given are the averages of the duplicates.

* Refer to p. 21 for expansion of abbreviations.

TABLE V. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT AND 80°F.[1/]

% of Surface Sterilized Grains Infected

Sample Description	*Fus.	A.fl.	A.ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor	No fungi
Grain Treet 0.6%	0	0	0	0	0	0	0	0	100
Grain Treet 0.7%	0	0	0	0	0	0	0	0	100
Grain Treet 0.8%	0	0	0	0	0	0	0	0	100
Grain Treet 0.9%	0	0	0	0	0	0	0	0	100
Kemin's Exp. Product 0.6%	76	94	0	0	0	0	0	0	0
Kemin's Exp. Product 0.7%	72	98	0	0	0	0	0	0	0
Kemin's Exp. Product 0.8%	72	100	0	2	0	4	0	0	0
Kemin's Exp. Product 0.9%	84	100	0	0	0	0	0	0	0

[1/] The data given above refers to the samples obtained from grain stored in cans under anaerobic conditions. The values given are the average of the duplicates.

* Refer to p. 21 for expansion of abbreviations.

TABLE VI. VISUAL APPEARANCE AND GERMINABILITY OF GRAIN AT THE END OF 30-DAY EXPERIMENTAL PERIOD

	Data from Storage in Cans		Data from Humidity	
Sample Description	Appearance at the end of 30 days*	% of germination	No. of days before the onset of mold	Appearance at the end of 30 days*
Control	+++	0	0	+++
Ethanol	+++	0	1	+++
Sorbic acid 0.1%	-	0	4	+++
Sorbic acid 0.2%	-	0	5	+++
Sorbic acid 0.3%	-	0	6	+++
Sorbic acid 0.4%	-	0	12	+++
Sorbic acid 0.5%	-	0	18	+
Propionic acid 1.0%	-	0	>30	-
Acetic acid 1.0%	++	0	5	+++
Calcium propionate 1.0%	++	0	6	+++
Kemin's Exp. Product 0.1%	++	0	2.5	+++
Kemin's Exp. Product 0.5%	++	0	3.5	+++
Grain Treet 0.1%	+	0	2.5	+++
Grain Treet 0.5%	+	0	12	++
Kemin's Exp. Product 1.0%	-	0	20	++
Grain Treet 1.0%	-	0	>30	-
Kemin's Exp. Product 0.6%	+	0	16.5	++
Kemin's Exp. Product 0.7%	+	0	20	++
Kemin's Exp. Product 0.8%	-	0	20	+
Kemin's Exp. Product 0.9%	-	0	20	+
Grain Treet 0.6%	-	0	>30	-
Grain Treet 0.7%	-	0	>30	-
Grain Treet 0.8%	-	0	>30	-
Grain Treet 0.9%		0	>30	-
Grain Savor (New) 0.1%	++	0	2	+++
Grain Savor (New) 0.2%	++	0	3	+++
Grain Savor (New) 0.3%	++	0	3	+++
Grain Savor (New) 0.4%	++	0	3.5	+++
Grain Savor (New) 0.5%	++	0	4	+++
Grain Savor (New) 1.0%	-	0	20	+

+++ Heavy mold infection, ++ Moderately infected, + Lightly infected
- No mold damage detected upon visual observation

*The data reported above are the averages of the duplicates.

TABLE VII. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT, 72°F. AND IN 100% RELATIVE HUMIDITY CHAMBER.[1/]

% of Surface Sterilized Grains Infected

Sample Description	*Fus.	A.fl.	A.ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor	No fungi
Control	96	90	16	0	0	0	32	12	0
Ethanol	40	100	20	4	6	0	6	12	0
Sorbic acid 0.1%	74	56	20	10	0	0	0	0	0
Sorbic acid 0.2%	36	74	90	12	0	2	0	0	0
Sorbic acid 0.3%	26	52	48	48	0	6	0	0	0
Sorbic acid 0.4%	16	12	50	0	0	0	0	0	44
Sorbic acid 0.5%	2	0	2	0	0	0	0	0	96
Propionic acid 1.0%	2	0	0	0	0	0	0	0	98
Acetic acid 1.0%	58	10	0	38	0	10	0	0	8
Calcium prop. 1.0%	40	50	0	0	0	2	0	0	30

[1/] The data reported are from the samples of the grain stored in relative humidity chambers.

* Refer to page 21 for expansion of abbreviations.

TABLE VIII. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT, 72°F. AND IN 100% RELATIVE HUMIDITY CHAMBER.[1/]

% of Surface Sterilized Grains Infected

Sample Description	*Fus.	A.fl.	A. ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor	No fungi
Kemin's Exp. Product 0.1%	38	96	58	0	0	0	26	34	0
Kemin's Exp. Product 0.5%	58	96	4	8	4	34	12	4	0
Grain Treet 0.1%	56	86	4	20	0	0	46	20	0
Grain Treet 0.5%	24	96	28	10	6	14	10	0	0
Kemin's Exp. Product 1.0%	22	44	46	2	0	6	0	0	42
Grain Treet 1.0%	4	0	0	0	0	0	0	0	96

[1/]The data reported here are from the samples of the grain stored in relative humidity chamber.

*Refer to page 21 for expansion of abbreviations.

TABLE IX. FUNGAL INVASION OF CORN STORED 30 DAYS AT APPROXIMATELY 26% MOISTURE CONTENT, 72°F. AND IN 100% RELATIVE HUMIDITY CHAMBER.[1/]

% of Surface Sterilized Grains Infected

Sample Description	*Fus.	A.fl.	A.ni.	A.gl.	A.ter.	Pen.	Rhi.	Mucor	No fungi
Kemin's Exp. Product 0.6%	40	98	44	8	0	68	2	0	0
Kemin's Exp. Product 0.7%	48	100	8	4	0	76	0	0	0
Kemin's Exp. Product 0.8%	18	70	2	2	0	30	0	0	4
Kemin's Exp. Product 0.9%	6	14	0	0	0	0	0	0	82
Grain Treet 0.6%	10	2	0	0	0	0	0	0	90
Grain Treet 0.7%	10	0	0	0	0	0	0	0	90
Grain Treet 0.8%	6	0	0	0	0	0	0	0	94
Grain Treet 0.9%	8	0	0	0	0	0	0	0	92
Grain Savor (New) 1.0%	16	100	0	8	0	20	0	0	0

[1/] The data reported here are from the samples of the grain stored in relative humidity chambers.

* Refer to page 21 for expansion of abbreviation.

TABLE X. EFFECTS OF PELLETING ON AFLATOXIN

Sample Description	Conc. of Aflatoxin (p.p.b.) B_1	B_2	G_1	G_2	pH
Batch I (No chemical added):					
Prior to conditioning	525	1200	225	157.5	
After conditioning	450	1050	202.5	135.0	
After pelleting	375	900	180	112.5	6.65
Batch II (2% NaOH added):					
Prior to conditioning	450	900	180	112.5	
After conditioning	375	900	180	112.5	
After pelleting	375	900	180	112.5	10.35
Batch III (2% Amm. hydroxide added):					
Prior to conditioning	525	1200	202.5	112.5	
After conditioning	450	1050	202.5	90.0	
After pelleting	450	900	202.5	90.0	7.52

TABLE XI. COMPOSITION OF FEED USED FOR PELLETING STUDIES

Description of the Ingredients	No. of pounds
Soybean oil meal	75
Ground yellow corn	125
Ground milo	120
Ground oats	50
Dehydrated alfalfa meal	25
Meat and bone meal	25
Fish meal	12.5
Shorts	50
Dicalcium Phosphate	10
Limestone	5
Salt	2.5
TOTAL	500.0

PLATE 1.

Fig. 1. A record of ambient temp. during the experimental period for the three sets of experiments. (Recorded daily at 12:00 a.m. simultaneous with the recording of the temp. of grain mass.)

Fig. 2. A comparison of heat-inhibiting activities of distilled water, propionic acid (1.0%) and acetic acid (1.0%) in high moisture corn (m.c.= 26-28%) storage.

Fig. 3. A comparison of heat-inhibiting activities of acetic acid (1.0%), and calcium propionate (1.0%) in high moisture corn (m.c.= 26-28%) storage.

$\Delta T^{\circ}F$ = Variation in the temp. of grain mass in comparison with ambient temp. in $^{\circ}F$.

T_A = Ambient temp. in $^{\circ}F$.

T_g = Temp. of the grain mass in $^{\circ}F$.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

FIG. 1

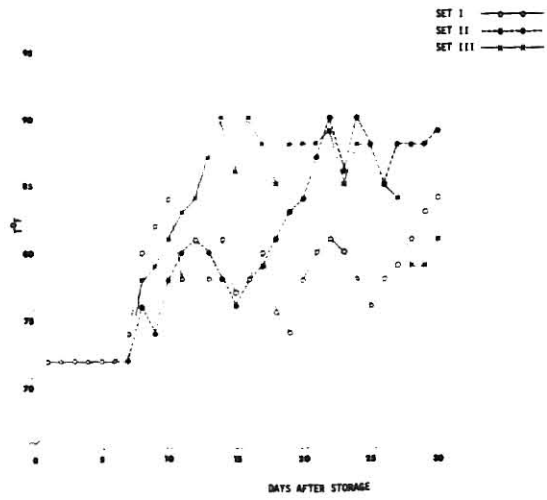


FIG. 2

FIG. 3

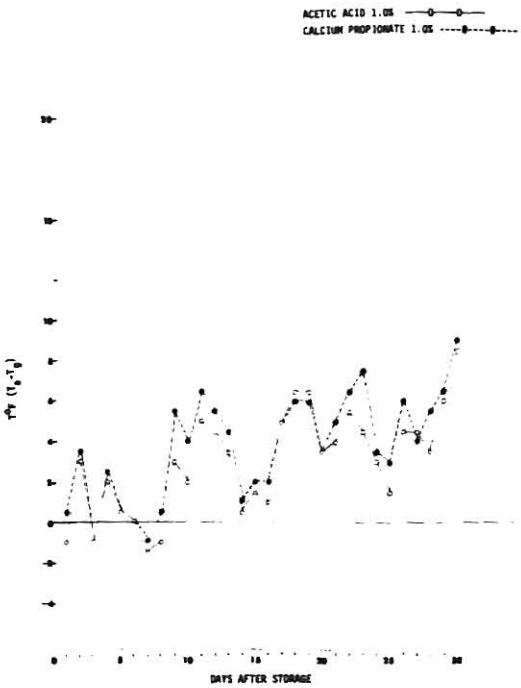


PLATE 2.

Fig. 4. A comparison of heat-inhibiting activities of ethanol (5.0%), and sorbic acid (0.1% vs. 0.5%) in high moisture corn (m.c. = 26-28%) storage.

Fig. 5. A comparison of heat-inhibiting activities of sorbic acid (0.1%), and propionic acid (1.0%) in high moisture corn (m.c. = 26-28%) storage.

Fig. 6. A comparison of heat-inhibiting activities of sorbic acid (0.5%) and propionic acid (1.0%) in high moisture corn (m.c. = 26-28%) storage.

Fig. 7. A comparison of heat-inhibiting activities of Kermin's Experimental Product (0.5%), Grain Treet (0.5%), and Grain Savor (0.5%) in high moisture corn (m.c. = 26-28%) storage.

$\Delta T^{\circ}F$ = Variation in the temperature of grain mass in comparison with ambient temperature in $^{\circ}F$.

T_A = Ambient temp. in $^{\circ}F$.

T_g = Temp. of the grain mass in $^{\circ}F$.

FIG. 4

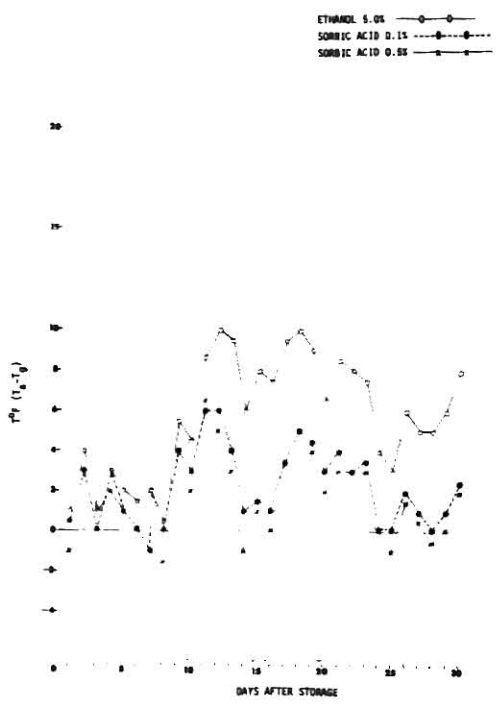
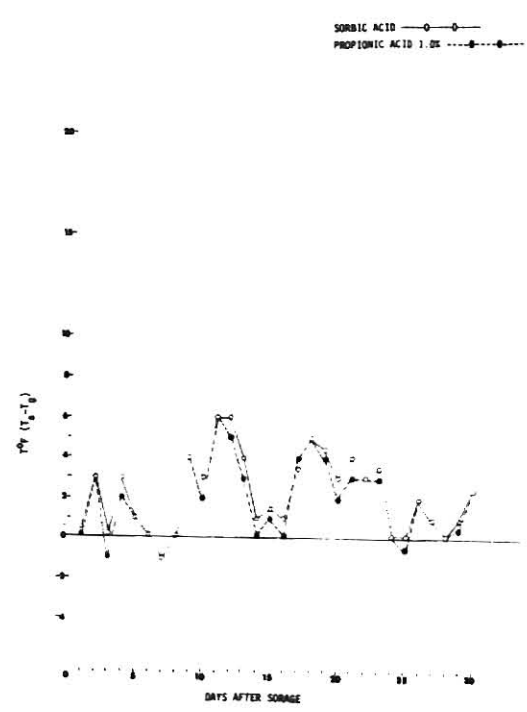


FIG. 5



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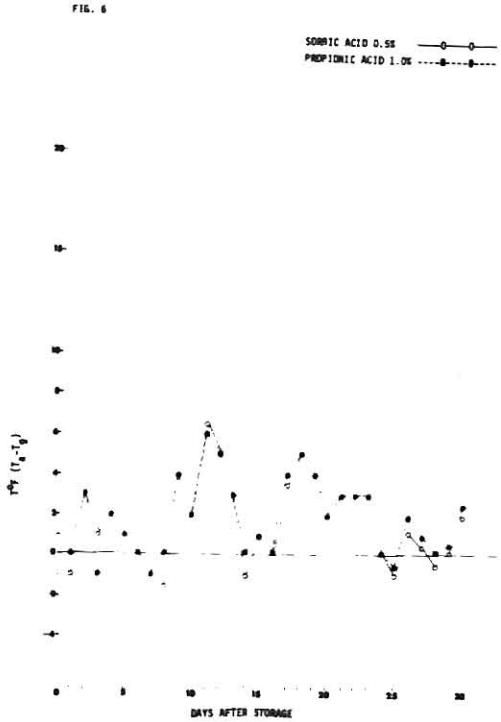


FIG. 7

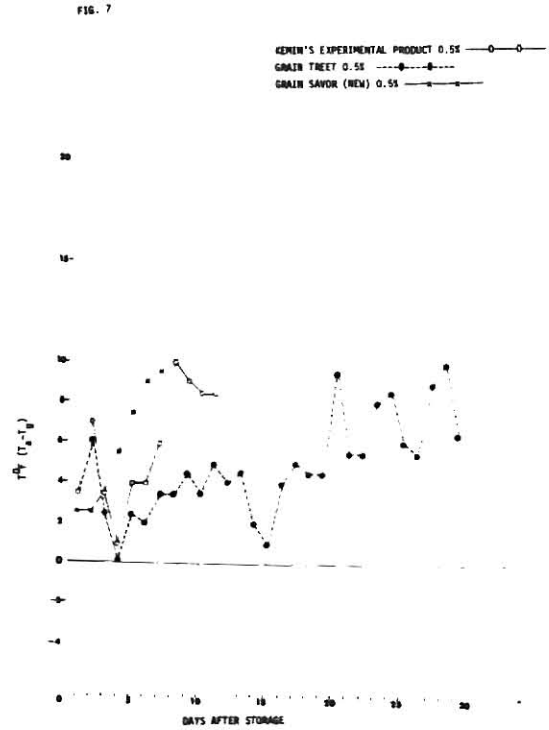


PLATE 3.

Fig. 8. A comparison of heat-inhibiting activities of Kemin's Experimental Product (1.0%), Grain Treet (1.0%) and Grain Savor (1.0%) in high moisture corn (m.c.=26-28%) storage.

Fig. 9. A comparison of heat-inhibiting activities of Grain Treet (0.6% vs. 1.0%), and propionic acid (1.0%) in high moisture corn (m.c.=26-28%) storage.

Fig. 10. A comparison of heat-inhibiting activities of Grain Treet (0.6% vs. 1.0%), and acetic acid (1.0%) in high moisture corn (m.c.=26-28%) storage.

Fig. 11. A comparison of heat-inhibiting activities of Grain Treet (0.6% vs. 1.0%), and calcium propionate (1.0%) in high moisture corn (m.c. = 26-28%) storage.

$\Delta T^{\circ}F$ = Variation in the temperature of grain mass in comparison with ambient temperature in $^{\circ}F$.

T_A = Ambient temp. in $^{\circ}F$.

T_g = Temp. of the grain mass in $^{\circ}F$.

FIG. 8

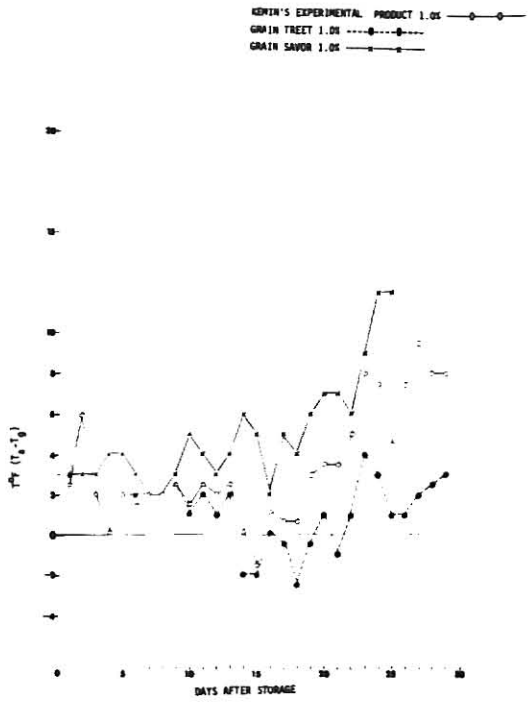


FIG. 9

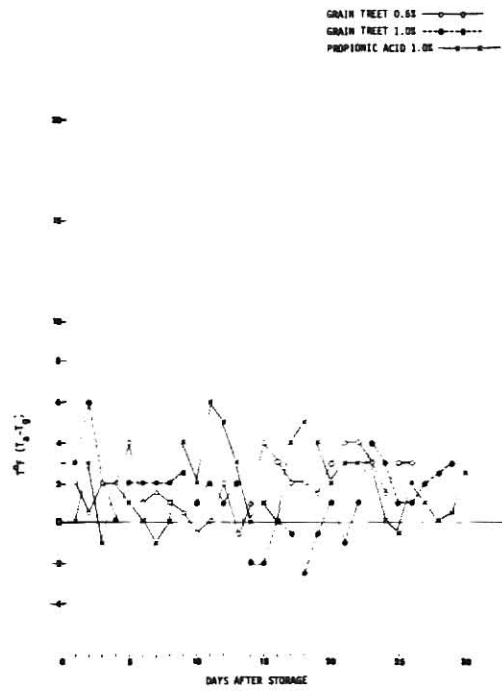


FIG. 10

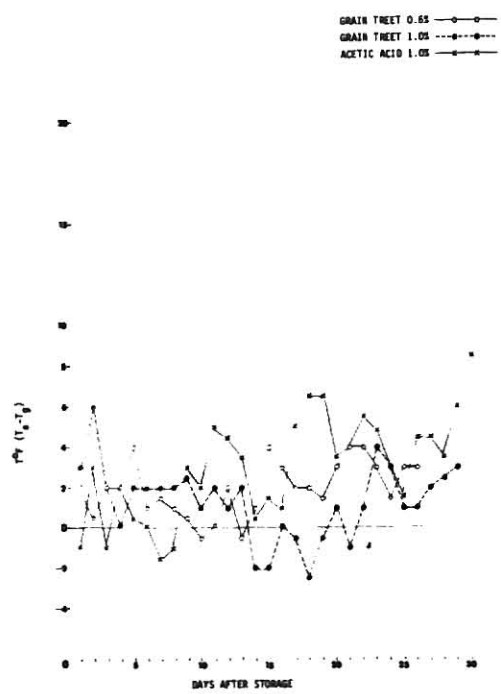
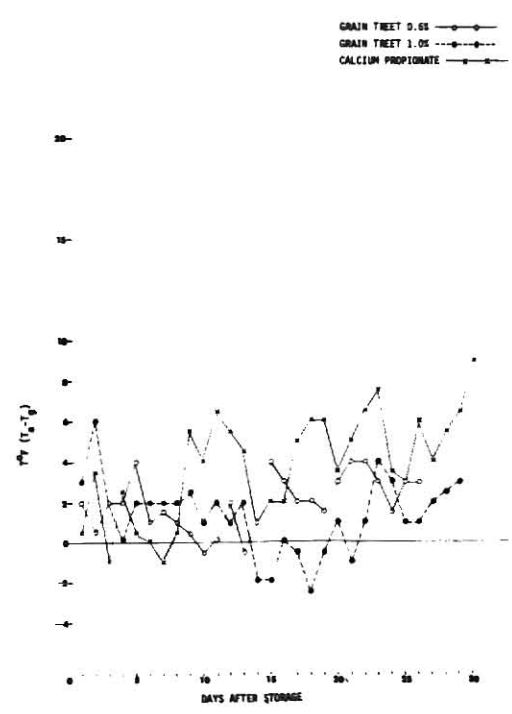


FIG. 11



SUMMARY

The influence of acetic acid, propionic acid, sorbic acid and calcium propionate on the storage of high moisture corn was studied. They were compared with the performance of Kemin's Experimental Product and Grain Treet (commercial products of Kemin Ind., Des Moines, Iowa). The parameters tested for the purpose were heat-inhibiting activity, time before the onset of moldiness in each treatment, and the kinds of fungi in the grain. Grain Treet was superior to all other chemicals tested.

Attempts to eliminate aflatoxin by way of pelleting alone, or with the addition of sodium hydroxide (2%) or ammonium hydroxide (2%) followed by pelleting did not produce significant beneficial effects. This was considered to be due to a short conditioning period and possible lack of sufficient time for interaction between the chemical and aflatoxin. It may also have been due to interaction between the chemicals and nonspecific feed ingredients.

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CHEMICAL PRESERVATION OF HIGH MOISTURE CORN AND EFFECTS OF PELLETING ON AFLATOXIN

by

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B.V.Sc., Kerala University, India, 1967

AN ABSTRACT OF MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

Kansas State University

Manhattan, Kansas

1972

The effects of sorbic acid, potassium sorbate, acetic acid, propionic acid, Kemin's Experimental Product, Grain Treet® and Grain Savor (New)® (the last three being products of Kemin Ind. Inc., Des Moines, Iowa) and calcium propionate at various levels on reconstituted high moisture corn (moisture content, approx. 26-28%) stored under laboratory conditions simulating aerobic and anaerobic systems, were studied and compared. The period of storage was 30 days. Temperature changes within the grain mass and the occurrence of various fungi at the end of trial period were recorded. Grain Treet and propionic acid were found to be most effective at levels of 0.6% and 1.0%, respectively.

In another study carried out using aflatoxin contaminated feed (K.S.U. Poultry Feed formulation), pelleting with or without the addition of 2% sodium hydroxide or 2% ammonium hydroxide did not significantly alter the aflatoxin content of feed.